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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

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Vol. VIII. December, 1904. No. 1

BIOLOGICAL BULLETIN

SOME NEW EVIDENCES FOR THE INDIVIDUALITY OF THE CHROMOSOMES.

W. J. BAUMGARTNER.

Introduction.

Owing to the renewed interest in hybridization aroused by the rediscovery of Mendel's laws, any facts that throw light on any of the theories used to explain the complex phenomena of the inheritance of specific characters in crosses must be welcome to biologists. In his résumé of the observations on hybrids and germ-cell generation Haecker (13) lays great stress on the theory of the individuality of the chromosomes. The same has been done by Sutton (31), Cannon (7), (8) and others. In this paper I propose to publish some observations made on the germ cells of crickets which furnish two lines of evidence establishing still better the individuality of the chromosome.

The first of these concerns the accessory chromosome in whose behavior I have found additional proof of its distinctness from the other chromosomes.

The second line of evidence concerns the ordinary chromosomes. Boveri (5) has recently found a difference *in function* in the chromosomes; Sutton (30) has found a difference *in size;* and I have been fortunate enough to find a difference *in form,* a characteristic shape assumed by the chromosomes in the prophase and metaphase of the first spermatocyte division.

Methods and Material.

As indicated in my former paper (3), Flemming's strong chromo-aceto-osmic fixative is best for the study of cricket germ-cells. Thirty-five per cent. alcohol saturated with corrosive sublimate plus ten per cent. acetic acid proved fairly good but material thus fixed shrank too much during imbedding.

The best stains were Heidenhain's iron-hæmatoxylin and Flemming's triple stain.

Besides the material used before, I have collected the common black field cricket about Chicago, and Woods Holl, Mass. The specimens from Lawrence, Kans., were called *Gryllus assimilis* after comparing them with specimens in the University collection there. Mr. F. E. Lutz called the same specimens *Gryllus luctuosus* after comparing them with forms about Chicago. I shall not enter into a discussion as to what are good species among *Gryllus* or what species are found in the different localities, but shall continue to use the name *Gryllus assimilis* for the species of the common black field cricket until the species are better differentiated.

The testes of the field cricket were described in the former paper and that of *Gryllus domesticus* corresponds very closely with it as to shape, size, location and arrangement of follicles and cysts.

The number of chromosomes in the field cricket is twenty-nine in the spermatogonia and not twenty-three as suggested in the first paper. Then I determined the number by counting the chromosomes in the equatorial plate of the first spermatocyte division and there the counting is difficult for the reason that the chromosomes usually do not enter the equatorial plate at the same time. I have made a great many counts since both in spermatogonia and spermatocytes and feel confident that the numbers are twenty-nine, and fourteen or fifteen respectively. In *G. domesticus* there are but twenty-one in the spermatogonia and ten or eleven in the spermatocytes. Because of this smaller number *G. domesticus* is more favorable for making drawings.

The stages with their relation and limits and the use of terms is indicated in the former paper. One point stated there I wish to emphasize as it is even more marked in *G. domesticus* than in *G. assimilis,* namely the fact that the cells in one cyst are not all in *exactly* the same stage of development. Some of the cells, usually on one side of the cyst, are a little in advance and others lag a little. This makes it possible to see the exact sequence of stages by comparing the cells of the cysts in different follicles.

Thus one cyst will have most cells in the metaphase but a few will be in early anaphase, while the neighboring cyst in the next follicle may have most of the cells in late anaphase but a few will be in early anaphase. The precocious cells in one cyst connect directly with the laggards of another. This is a great advantage for correct interpretation of the appearances.

OBSERVATIONS.

The accessory chromosome appears in the earliest secondary spermatogonial divisions. During mitosis it takes its place in the periphery of the equatorial plate (Figs. 1 and 2). It always has a bend at the center, and the whole is in the shape of an irregular horseshoe (Fig. 1) or its two ends are spread out almost straight so that it is a straight rod with a short semicircle-like bend at the center (Fig. 2). As the other chromosomes split and pass to the poles the accessory also splits longitudinally (Fig. 3). The curves of the horseshoe-like rods separate first and the ends of the two daughter chromosomes are the last part to be separated, as indicated by McClung (16), in *Xiphidium*. All through the metaphase and anaphase the accessory is quite a little behind the others. Its ends are sometimes scarcely separated when the other chromosomes have reached the poles. During the rest stage it does not become granular and disappear like the others but remains a darkly staining mass.

In the early growth period while the other chromosomes become diffuse, the accessory takes its position against the wall of the nucleus. Here it lies a strongly staining mass adherent to the one side. Through all the long growth period the accessory can be distinguished from the nucleolus by its stain for a part of the time (Fig. 10), but it can be recognized *all the time* by its position as it lies flattened against the outer wall. The nucleolus, which for a part of the growth period takes the same stains as does the accessory and just as readily, can usually be distinguished by its rounded or oval shape. Its position in the nucleus varies with almost every cell. It may be in the center or near the periphery, opposite the accessory (Figs. 4, 5 and 8) or near to it (Figs. 6 and 7). It often lies against the accessory or even partly over it (Figs. 6 and 10). This fact led

Voinov (32) to describe the two closely approximated bodies as a double nucleinic body — "corps nucleinien double." (For a criticism see in "Discussion" below.) I could note no difference in the size of the nucleolus that was constant. In the prophase of the first spermatocyte division the nucleolus gradually loses its ability to stain and disappears completely while the chromosomes are forming.

The accessory chromosome can be distinguished from the ordinary chromosomes in late prophase by its denser stain and smooth outline. But as the chromatin concentrates more and more in the latter we must find other criteria. The position no longer answers, as many of the ordinary chromosomes now come to lie in or near the periphery of the nucleus, and in a few cases it becomes something of a puzzle to know which is the accessory. But about this time the shape assumed by the latter becomes a mark of distinction from the others. As shown in Figs. 12, 13, 15, 17, and 20 it assumes a sausage shape and keeps it all through the first spermatocyte division. As the spindle is formed and the other chromosomes are drawn toward the equatorial plate, the accessory may be in any position whatever within the nucleus. McClung (18) says it appears nearer one pole. It does very frequently. It may be within the spindle or out in the cytoplasm as Sinéty (28) thought he found it in other Orthoptera. But it may lie even in the equatorial plate where I have seen it in several cases. Either the open or the curved side of "the sausage" may point toward its nearer pole. In metaphase and anaphase it is usually the curved side.

As the other chromosomes move to the poles the accessory also seems to be drawn towards its nearer pole but it is again a laggard (Fig. 23). It does not divide but passes entire to one of the poles as Sinéty has already indicated for *Gryllus campestris* and he and McClung have described it in Locustids and Phasmids. I found a few cases where the accessory was left in the plane of cleavage between the two daughter cells. Here it was divided into two equal parts as seen in several cases (Fig. 22), and unequal in one case. In one case the accessory was just in the center between two poles and showed constrictions at its center (Fig. 21). In these cases I believe the accessory happened to lie in the region of the equatorial plate and neither pole was able to attract it, and so being left in the region of the new cell wall was constricted mechanically. I do not think that this division is the same as that of normal cleavage of the accessory as it occurs in the second spermatocyte division.

The following fact in the behavior of the accessory has not been recorded heretofore by any investigator as far as I know. It throws some additional light on its own independence and hence on the individuality of the chromosomes.

As recorded by most writers on insect spermatogenesis there is no resting stage or no *true* resting stage between the first and the second spermatocyte divisions. But I have found in *Gryllus* what I shall call a *semiresting stage* following Katharine Foot's (11) terminology. She has described a stage in the *Allolobophora* egg to which my own findings correspond in many particulars. At the close of the anaphase of the first division the chromosomes are crowded around the pole. The centrosome divides about the time or a little before the chromosomes reach the pole. The two centrosomes begin to move apart with the radiating fibers of the asters around them individually and the spindle fibers connecting them (Figs. 23 and 24). As the spindle is elongating the chromosomes become somewhat vesicular (Figs. 25 and 26) and have a nuclear wall formed around them, entirely around or only part way around (Figs. 26 and 27). The diffusion of the chromatin and the formation of the nuclear wall seems to go farther in some cells than in others; Fig. 27 shows as much diffusion as any observed. The whole semiresting stage must be very brief for a single cyst may show cells as far apart in development as shown in Figs. 24 and 30. As the chromosomes enter the equatorial plate of the second division they are usually so crowded that it is impossible to count them or distinguish the accessory from the others.

After this brief description of the semiresting stage let us return to the accessory chromosome.

In the semiresting stage the accessory does not enter into the nucleus but forms its own wall around itself. It becomes vesicular, the chromatin becoming granular and showing vacuoles (Figs. 24–27). Its position with relation to the nucleus varies.

Very frequently it lies just outside the nucleus so that there seems to be but one dividing membrane between the two (Fig. 25). It may be entirely away from the nucleus showing the two walls very distinctly (Fig. 26). In one case I found the spindle between the accessory vesicle and the nucleus (Fig. 24). In other cases the accessory lies entirely in or partly in the other nucleus (Fig. 27); but in every case *it has its own vesicular wall around itself, and does not form part of the nucleus.* Of course, the accessory is found in only one half the cells.

When the second spermatocyte spindle is formed the accessory enters the equatorial plate and can not always be distinguished because of the crowding of the chromosomes spoken of above (Figs. 28 and 29). In a few cases I could distinguish the individual ones and count them (Fig. 31). Here the accessory is marked by its size and characteristic shape. But as soon as all the chromosomes divide and move apart in anaphase, they can be counted in almost every cell. The accessory again lags behind but usually not so much as in the earlier stages. Figs. 32 and 33 give side views and Figs. 34 and 35 almost polar views of the anaphase stage; Figs. 32 and 34 show the accessory and Figs. 33 and 35 lack it. Figs. 34 and 35 are very interesting because they were found side by side in a cyst and indicate the absence of the accessory in one half and its presence in the other half of the cells. The accessory in the telophases and in the spermatids has been described in my former paper to which readers are referred for its farther history.

The Ordinary Chromosomes.

While the above description of the accessory applies equally well to either species of *Gryllus*, what follows applies only to *G. domesticus*. *G. assimilas* shows some differences of size and shape in the ordinary[1] chromosomes, but it is not at all marked.

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[1] At first I was inclined to adopt Montgomery's (26) terms homochromosome and heterochromosome to distinguish between the ordinary chromosomes and the accessory. But upon reflection it seemed to me it would lead to confusion with the use of the words heterotypic and homotypic. If the terms were adopted then we should have both homochromosomes and heterochromosomes in the homotypic division as well as in the heterotypic. This mixing of terms seems undesirable.

There is here one frequently showing an L shape somewhat as Sinéty (28) has described in a Phasmid, but this is not the accessory joined to an ordinary chromosome as Sinéty interpreted his figure.

In *G. domesticus* in the spermatogonia there are twenty ordinary chromosomes as shown in Figs. 1 and 2. These show differences of size and also in amount of curving. The short ones are straight or almost so, and the long ones are curved, some more, some less. The amount of curving does not depend on the length only as can be seen by comparing chromosome pairs 5 and 6 in Fig. 2. The chromosomes can be arranged into a graded series of pairs following Sutton (30) and Montgomery (25) but the differences between certain pairs is often very slight so that one could often as well make groups of three's or four's. In Fig. 2 I have made an attempt to bring together the probable pairs, but in many cases the arrangement is very unsatisfactory. Of course, looking at the chromosomes themselves with adjusting focus relations are more apparent than can be indicated by a camera drawing. In pairs one and two of Fig. 2 the two chromosomes on the right side of the respective pairs may belong together to form one pair, and the two on the left the other. Grouping them as suggested would bring together chromosomes that do not differ more than do some of the chromosomes of the pairs as they now stand, *e. g.*, pair 3.

After the spermatogonial divisions are completed the cells enter the growth period. The chromosomes seem at first to break up and the chromatin apparently becomes diffuse, yet it appears partly in threads as shown in Fig. 4. I have looked very long and carefully for a massing of the chromatin thread into one part of the nucleus hoping thus to find what Montgomery (22) had described as the "synapsis stage" I was unable to find anything that corresponded with his description or drawings, although he named *Gryllus* as one of the forms in which he saw the synapsis stage. But upon studying the ovocytes during the growth period I found many cells showing the conditions indicated in Fig. 36. This drawing is made from an ovary taken from *Scapteriscus didactylus*, a Porto Rican mole cricket, kindly sent me by O. W. Barrett, of the agricultural experiment station. Both species of *Gryllus* show the same conditions. Many of the other cells show the loops more crowded, especially the younger (smaller) ovocytes while the older (larger) ones show them looser.

With the idea in mind that the chromatin thread is in loops I succeeded in finding Fig. 5 in the early spermatocytes. This figure shows the loops as much massed leaving as much of the nucleus clear as any case I have observed. In most cells the loops fill the nucleus completely ; besides the large majority of the cells are so placed in the field that they show nothing of the looping of the thread. This I consider the characteristic of the "synapsis stage" in the testis of *Gryllus* and not the massing to one part of the nucleus as Montgomery did in his work on *Pentatoma.*

This stage appears quite early in the growth period and lasts a comparatively long time. All the cysts from which Figs. 5, 6, 7 and 8 are drawn show some cells where the loops of the chromosomes are quite evident, but most cells show little or nothing of such loops owing to the plane of cutting, as indicated in the figures.

After this stage the chromatin becomes a little more diffuse again before forming the definite chromosomes of the first spermatocyte division (Fig. 10). We shall now pass to the metaphase where the chromosomes are completely formed and lie in the equatorial plate region and return to the late prophases subsequently.

A glance at Fig. 18 will give the reader an idea of the various forms of chromosomes seen in a cyst full of cells in this stage. Drawings of all the forms that could be found showed the possibility of classifying the shapes into rings, crosses and rods. The rod may be straight, or bent so as to form a bracket, parenthesis or an *S.* The crosses may have the ends bent so as form an *f* or an ϵ where one arm is very short or absent. All these shapes can be seen in Figs. 13–19. It was observed that many cells showed two rings (Figs. 13 and 18, *a*) and one or two crosses and several rods some of which were straight. If there were many straight rods fewer other shapes appeared. Some showed no rings others no crosses. Naturally the question arose : is there any constancy in the number of chromosomes in a cell that assume a certain shape ?

To test this I sketched twenty-one groups of chromosomes that showed a variety of shapes and classified them into rings, crosses, rods, brackets, parenthesis, *f*'s, *S*'s and ϵ's. I found that but two groups showed no rings and eleven groups showed two rings, but in no case were there more than two. Five cases showed no crosses and nine cases showed two.

It is evident that any one of the shapes represented may be turned in such a way in the cell that it would appear as a straight rod. This might account for the non-appearance of the rings, or any of the other forms in my sketches. I then tried the hypothesis that there were *two rings* in each cell and tested it as follows :

To find out what per cent. of rings should appear I proceeded thus. The ring is formed of a cylindrical rod. Measuring a number of rings with the eye-piece micronometer I found that the opening was about one third of the diameter. I then moulded a ring of modeling clay making the rim of a rod whose diameter was equal to that of the central opening. By turning this doughnut-like ring on a sheet of paper it was found that the opening was not visible for about 67° of the semicircle. Sixty-seven and one half degrees is three-eighths of a semicircle, hence three rings out of every eight should not show any opening. But as the ring becomes wide enough to be distinguished as a ring before the opening can be seen, it seemed possible to estimate the percentage of rings that should be recognizable. By using the above ring of clay and testing several of the co-workers in the laboratory as to the angle at which they could be reasonably sure that it was a ring and not a rod they were seeing it was found that on an average the ring could not be recognized for fourty-five degrees of the semicircle. Fourty-five degrees is one-fourth of one hundred and eighty degrees, or one ring in four should not be recognizable.

Of the twenty-one sketched groups ten showed all the ordinary chromosomes. If there are two rings in each cell, according to the hypothesis there should be 20 present, of which 15 should be recognizable. My table shows 13.

I then sketched 25 cells, all showing the full number of chromosomes, 10. These should have 50 rings, three-fourths of which, or 37½, should appear. After completing my table I counted the number of rings and found 35 with six marked doubtful. If we count one half of these we get 38, or just the required number. This does not prove that there are always two chromosomes that assume the ring shape, but it makes it probable.

A few facts can be added from the prophase stages that increase the probability that these shapes are characteristic of the individual chromosomes. Fig. 11 is a careful drawing made of a cell in a late prophase. The nuclear wall is still intact. The accessory is densely stained and smooth in contour. The ordinary chromosomes are not yet compact or smooth in outline, but they show many of the shapes appearing later. Counting the chromosomes marked *6*, which was drawn from the next section and carefully identified as belonging to this cell, there are the two rings, two crosses and several curved and bent rods. Fig. 8 shows a well-formed ring in an earlier stage. The formation of the tetrads has not been studied in minute detail, since this has been done by McClung in the Acrididæ (17) and Locustidæ (18), more favorable material for this part of the problem. Fig. 9 shows the chromatin rod with the longitudinal split indicated.

Fig. 12 gives a polar view. The nuclear wall has just broken down and the chromosomes have not been drawn into the equatorial plate. Here both ring chromosomes are visible which is unusual for a polar view. Some of the other shapes can be seen. I think that the chromosomes are not yet drawn by the fibers and hence show these shapes from this view. Figs. 13, 14 and 16 and *a*, *b*, *c* in 18 show the diversity of forms that can be made out in a single nucleus. The varied shapes can best be seen in a nucleus just after the nuclear wall has broken down and the chromosomes are being drawn toward the center. Fig. 13 and 18, *a*, are taken from cells in this stage.

In Fig. 19 we have a drawing made from a smear preparation. Again appear two rings, straight and bent crosses and rods. The chromosome marked *u* has its ends crossed and thus forms a modified ring. I think that this is a result of the pressure used in spreading the cells on the cover, as I saw it on no other slide. While the smear method shows the chromosomes well, in none of my preparations so made does the spindle appear and so it is difficult to fix the exact stage of development of the cell. The chromosomes in most smear preparations were irregularly distributed in the cell, yet a few showed them in a kind of equatorial plate. I can confirm what Sinéty (28) has said about the metaphase: "A parlèr rigoureusement le métaphase néiste pas dans cette catégorie de cellules" (first spermatocyte). In many cells of first spermatocyte there is really not a metaphase, that is, there is no stage in which all the chromosomes lie in one plane at the equator of the spindle, but as some are entering others have separated and are passing to the poles. This is partly illustrated by Fig. 17, but others of my drawings which I have not been able to put among these figures show this difference of arrival at the equatorial plate region very distinctly. Some cells show a good metaphase (Fig. 14).

Fig. 16 and 17 show the division of the ring which breaks into two semicircles at the equatorial plane. Fig. 17 shows the *u*, or *v*, shaped chromosomes described by so many writers in the anaphase of the first maturation division.

The ordinary chromosomes in the semiresting stage were described above while speaking of the accessory.

Attention should be called to the difference of shape of the chromosomes as they appear in Figs. 31 and 34 or 35. While in the former we have the bent rods quite numerous, in the latter they are mostly straight rods. The spermatogonia are curved less than those in the second spermatocyte and more than those of the spermatid.

COMPARISON OF RESULTS AND DISCUSSION OF LITERATURE.

As indicated above Sinéty (28) and McClung (18) have described and discussed the behavior of the accessory chromosome in the spermatocytes of the Orthoptera. Both papers appeared after my own principal results were obtained. I can hence add independent, confirmatory evidence of its failure to divide in the first spermatocyte mitosis and its division in the second and the resulting distribution to only two of the four spermatids. I have not been able to confirm McClung's observation of a spireme condition of the accessory in the growth period.

From the observation of the peculiar L chromosome in *G.*

assimilis, I very much question the correctness of Sinéty's interpretation of similar appearances in *Leptynia*. But my own results show too great differences in detail between species of one genus for me to *deny* the results of another worker on a different family without seeing the form he studied.[1]

I can add the following new facts concerning the behavior of the accessory chromosome. In the first spermatocyte spindle I have a greater variation in the position in which the accessory may be found. Beside the positions described by the above writers it may be in the equatorial plate region and as I believe constricted by the cleavage of the cell. That it is not a normal division of the accessory chromosome is proven by the fact that the parts separated may be unequal. It is simply a mechanical separation of the chromatin mass.

The behavior of the accessory chromosome in the semiresting stage is parallel to conditions which Sutton (29) has described in the spermatogonia of *Brachystola*. Sutton finds that the accessory always has its own vesicular wall and does not form a part of the regular nucleus. In *Gryllus* the accessory has its own vesicle in the semiresting stage between the first and second spermatocytes. The accessory here shows its independence in the part of the germcycle in which it has not been described, and so it offers additional proof of its own individuality, hence of *the individuality* of the chromosomes.

In the variety of shapes found in the first spermatocytes I do not claim to have anything new. But in interpreting the different forms as distinctive of individual chromosomes, I have assumed *a new view point*. Anyone following the investigations on germcells since Weismann (33) postulated a reduction division and Flemming (10) distinguished between heterotypic and homotypic mitoses will find a great number of shapes of chromosomes described. Some of these are characteristic of certain species. Other species have a great variety of shapes in the same cell. The efforts of the workers who have found these different shapes have been directed toward reducing them to a single type or form, or to show that all accomplish the same purpose and that the variations are only chance differences or certain stages in the development of the tetrad or stages in the first spermatocyte division.

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[1] Dr. McClung informs me privately, since the above was written, that he has found appearances in an Acrididæ which indicate that the accessory chromosome may unite with an ordinary chromosome forming an L-shaped mass; and so Sinéty's interpretation is probably correct. But the accessory *is not* a part of the L-shaped chromosome in *G. assimilis*.

It is impossible to discuss within the limits of this paper all the works that have described and discussed a variety of chromosome forms in the spermatocytes and ovocytes. For such discussions I would refer the reader to Wilson's "Cell," p. 264 ff., and Korschelt and Heider's "Entwickelungsgeschichte," p. 572 ff.

Wilson's interpretation of these different shapes is evident from this quotation: "But even in cases where the chromatin does not condense into actual tetrads these bodies are represented by chromosomes in the form of rings, crosses and the like, which are closely similar and doubtless equivalent to those from which actual tetrads arise, and present us with the same problems. With a few apparent acceptions described hereafter, the tetrads, or their equivalents, always arise by a double division of a single primary chromatin rod or mass." Wilson then discusses the various maturation divisions and chromosome shapes under:

(*a*) "Tetrad formation with one longitudinal and one transverse division," naming Henking's, Vom Rath's, Haecker's, *et al.* results.

(*b*) "Tetrad formation with two longitudinal divisions," Van Beneden and Boveri on Ascaris.

(*c*) "Tetrad formation by conjugation," Wilcox and Calkins especially.

In these cases the rings, etc., occur in the prophases.

"Reduction without tetrad formation" occurs where there is no resting stage and here "the equivalents of tetrads," the rings, crosses, etc., appear. Again, the divisions are longitudinal and transverse, found mostly in Invertebrates, or double longitudinal, found mostly in Vertebrates and in many plants.

Korschelt and Heider classify the various mitoses under "*eumitotic*," or double longitudinal, and "*pseudomitotic*," or one longitudinal and one transverse — a reduction division. After discussing very many papers giving the various forms of chromosomes described, and the interpretation that has been put on them, they make the suggestion (p. 591) that many of the chromosome forms are artefacts, "dass es sich bei manchen von ihnen um Kunstproduckte handeln möchte, wie sie durch die Conservirung hervorgerufen werden."

From the two excellent reviews of the literature referred to above it will be seen that all attempts at an explanation of the various shapes aimed at proving that there were two longitudinal splits of the chromosomes and hence two equational divisions, or a longitudinal split and a transverse split, hence an equational and a reductional division. Reduction is the question around which the whole discussion centers.

Montgomery's (25) observations on the salamanders show that in these forms there is a side-to-side union of the chromosomes in synapsis. The Schreiners (27) have observed the same fact in some of the lower fishes. If this proves true for the vertebrates in general, and if Farmer and Moore are correct in interpreting one of the longitudinal splits in plants as the line of union of two chromosomes, then the contradiction between Korschelt and Heider's eumitotic and pseudomitotic division is removed. The question as to which is the equation and which is reduction division has largely lost its importance, not because they have lost their significance as Wilcox (34) put forth, but because it is shown that there is an equation and a reduction division whatever may be the appearance of the chromosome in the prophase of the first maturation division (see Montgomery (26)).

I wish now, after having referred to the above general reviews, to discuss several papers more in detail because of their interest from my special view point.

Griffin's (12) work is taken by Korschelt and Heider (14) as a typical case of their "post-reductional" divisions. He gives careful descriptions of minute details. In the prophase he figures fourteen different shapes in the text and describes them as "rings, crosses, double rods, and apparently homogeneous rods variously coiled and bent." On page 607 he says: "Despite the varied forms presented during the prophase the chromosomes of the equatorial plate exhibit considerable uniformity. Hence the various prophase forms must in some manner be convertible into a uniform type of metaphase figure." Then in the metaphase he tries to reduce all to the cross type. But I do not think his drawings bear out his contention (see his drawings of *Thallasema* Figs. 12, 13 and 14 of Plate XXXI). Especially in Fig. 13 I do not see how he will get all to the cross type.

We have the following recent papers on the Orthoptera: McClung (17) indicates various shapes in his figures of the early prophases and says: "But despite the multiplicity of their forms these precursors of the chromosomes are all referable to a common type." This is a doubly split rod. Crosses in prophases are the result of the gliding together of the chromatids, or parts of the split rod. As a result of the concentration of the chromatin "the chromosomes in the nuclear plate appear to be simple homogeneous bodies." But they have the shape of rods, crosses, v's and rings. These McClung explains as the result of different views of the crosses, and the gliding of the chromatids or the bending of the arms of the crosses.

The interesting fact here is that while Griffin and most of the earlier writers tried to find that the chromosomes in metaphase either were all the same shape or could be reduced to the same type, McClung finds the various shapes in the metaphase, and rings divide as rings and crosses as crosses.

McClung (18) describes rings, crosses, figure 8, etc., in the prophase in the Locustidæ: "After concentration, while all trace of internal structure" (chromomeres and splits) "are gone, the general outline is retained and the crosses and rings of the early stages are still even up to the metaphase crosses and rings."

Sinéty figures a great variety in the Phasmidæ, Locustidæ, Acrididæ and Gryllidæ. The different shapes in the metaphase he explains by the different manner of insertion of the mantle fibers. This may be "median, subterminal or terminal." But this gives no reason for their shapes in the prophases.

Schreiner (27), A. and K. E., have described very recently in a hag-fish, *Myxine glutinosa*, and a dog-fish, *Spinax niger*, various forms of chromosomes in the prophases. In *Myxine* the chromosomes are concentrated into round or polygonal bodies, but "in a few cases they show in this stage plainly a ring form."

There is a pair of very large chromosomes in the spermatogonia and a single very large one in the spermatocyte. In *Spinax* there are graded size differences of chromosomes which they compare with Montgomery's and Sutton's results. They think the number of large chromosomes and small ones is constant but have not proven this. The large chromosomes form rings. The small ones form before mitosis mostly rods, seldom rings—"häufig Stäbchen, selten Ringe." In the equatorial plate the chromosomes retain their shape.

Here we have then from the recent papers, McClung, Sinéty and Schreiner, evidence that the shapes found in the prophases are still found in the metaphase. Their drawings do not show as many different forms in the metaphase as in the prophases, but some are probably hidden. In *Gryllus* all the various shapes in a cell are seen to best advantage in a late prophase, but I believe all the shapes are still to be seen in metaphase. A careful comparison of my Figs. 11 and 12, late prophases, with Figs. 14 and 16, metaphase will prove that the same shapes are found in earlier and later stages. The different kinds of attachment of the mantle fibers can not account for different forms in the prophases, although it may in the metaphase. Fig. 11 is very instructive. The chromosomes are still granular and rough in contour yet we find two rings, the number probably found constantly, and two crosses, a number frequently observed.

That these various shapes are artefacts as Korschelt and Heider (14) suggest, is made very doubtful by the fact that they are shown by my material fixed in very different reagents. They are shown in my sections and in my smear preparations. Besides the great army of investigators have used all kinds of fixatives and yet have found them.

That they are not simply stages through which the chromatin must pass to get into the *v* or horseshoe shape of the anaphase as Lebrun (15) has assumed, is combated by the long continuation of the same form from the early prophase to the anaphase. To reach such an end would need only *one* type of chromosome form in a single nucleus at any one time. This is contrary to the observation of the majority of workers who have observed chromosome differences.

That the shape is not a mere happening so, a ring now, and a cross then, is met, in part at least, by the probability of the constancy of the number of rings found in the various cells. I could see no way of testing the constancy of the other forms, such as the cross.

Taking all these observations into consideration it seems to me the best hypothesis that we can propose to explain the various forms assumed by the chromosomes in the prophases and metaphase of the first spermatocyte is to say *that these various shapes are an expression of the individual characteristics of the various chromosomes.* They are a proof of the individuality of the chromosomes. I would repeat in the way of emphasis that Boveri has found a difference *in function*, Sutton a difference *in size* and I a difference *in form* in the chromosomes of the germ cells.

That these observations will be found to have a wider application I would predict from the many indications I find in literature especially the results on *Ascaris.* But in many species as in *G. assimilis* the form of the chromosomes is not varied enough to establish a constancy of any one form, just as size differences can probably not be found in all species. Some other species will probably prove much better to establish morphological differences in the chromosomes than *G. domesticus*; yet I consider this material very good for the purpose. I trust that other workers who find a great variety of chromosome shapes will examine their material from this view point.

That the hypothesis, or theory as Boveri (6) would call it, has received an unfortunate name Wilson points out in his "Cell," p. 299. But I do not believe that many cytologists think that chromosomes persist as individuals. Nor do I believe that we generally think of a "continuity of the chromatic substance" as Haecker (13) supposes (see page 217). Nor is he the first to suggest that the continuity may rest upon the achromatic substance as shown in the quotation below.

This passage quoted from Boveri (4) I think gives an idea of what the advocates of the hypothesis mean by the individuality of the chromosomes. "Ich habe dieselbe als die Hypothese von der Individualität der Chromosomen bezeichnet, weil die Gebilde, die wir als selbstständige Stücke kennen, den Namen

"Chromosomen" führen, und die nächstliegende Annahme war nach den Befunden von Rabl und mir in der That die, dass jedes Chromosoma als solches in ruhendem Kern fortbestehe und nur seine form verändere. In letzter Instanz aber fordert die Hypothese nichts anderes als einen genetishen Zusammenhang zwischen je einem der aus dem ruhenden Kern hervorgehenden Elemente mit einem bestimmten der in die Bildung des Kernes eingegangenen. Was von dem Chromosoma als selbstständiges Gebilde übrig bleibt, ist für die Hypothese an und für sich gleichgiltig. Es mag unser hypothetisches Individuum z. B. die färbbare Substanz völlig verlieren und sich erst wieder bei der nächsten Teilung mit ihr beladen; ja es mag in gewissen Zellen nur ein mit unseren Mitteln gar nicht nachweisbares Teilchen von jedem Chromosoma übrig bleiben um als Bildungscentrum zur Entstehung der neuen Chromatin schleife Veranlassung zu geben: jedenfalls ist die Annahme eines genetishen Zusammenhanges je eines bestimmsten Chromatinsegmentes mit einem bestimmten der vorher sichtbaren die weitaus bestbegründete Annahme zur Erklärung aller in Betracht kommenden Erscheinungen und vor allem der bei den Kernteilungen zu beobachtenden normalen und abnormalen Zahlenverhaltnisse." It is the genetic relation of which most of us think when speaking of chromosome individuality. Such an individuality would be supported by the constancy of the appearance of a certain shape in a bivalent chromosome. If two pairs of chromosomes after conjugation form rings in every cell of a first spermatocyte generation they will form them in succeeding first spermatocytes and that means that there is a genetic relation.

It is not wise to theorize much on the importance of a hypothesis based on so few observations, but a few words indicating its meaning might not be amiss.

A constant morphological difference would strongly support Boveri (5) and Sutton (31) in their surmise that the chromosomes play different rôles in development. Sutton (31) makes this statement: "There is reason to believe that the division products of a given chromosome in *Brachystola* maintain in their respective series the same size relation as did the parent element; and this taken together with the evidence that the various chro-

mosomes of the series represent distinctive potentialities, make it probable that a given size-relation is characteristic of the physical basis of a definite set of characters." In the above sentence I should like to substitute *form* for "size-relation" and *Gryllus* for *Brachystola*. A difference in the shape of the chromosomes indicates the fact that they form the physical basis of different sets of characters. A difference of shape supports Boveri's supposition of a difference of "rôle of chromosome" even more strongly than a difference of size. But Boveri's method of study, multipolar spindles, can not be applied to Orthoptera. To me it seems probable that the test, if we can get any, must come from hybrid germ cells. Sutton (31) has shown that "the phenomena of germ-cell division and of heredity" as expressed by Mendel's laws "are seen to have the same essential features." Moenkhouse's (21) and Metcalf's (20) results teach us that forms with differently shaped chromosomes can be crossed. If now we can raise such hybrids to sexual maturity, we can probably get light on the "purity of the germ-cells" as well as on the meaning of the various shapes of the chromosomes.

Montgomery (26) criticizes Sutton (31) and says that the combinations of the paternal and maternal chromosomes in the fertilized egg do not follow the Mendelian ratio. In sustaining this position Montgomery says the Mendelian ratio can hold only in cases where there are but two chromosomes in the fertilized egg. For the case of four chromosomes the relation would be 1 : 14 : 1 instead of 1 : 2 : 1, and for twenty-four chromosomes 1 : 16,777,214 : 1. Montgomery must have entirely misunderstood Sutton or the Mendelian principle. Mendel found that *a pair of alternative characters* followed in self-fertilized hybrids the ratio of 1 : 2 : 1. Sutton found the same ratio for a *pair* of homologous chromosomes. Sutton also found in a form that had twenty-four chromosomes there could be 16,777,214 different combinations of chromosomes in the gamete. To take a concrete example in hybrids having twenty-four chromosomes a pair of alternative characters ought to appear in the second generation hybrid in the proportion 1D : 2Dr : 1r, but only one out of 16,777,214 of such hybrids should show *all the characters* belonging to one of the pure ancestors only and none of those of the other. Probably Dr.

Montgomery meant to explain his position and correct the error, when in his criticism of Haecker in the Zool. Anz. of June 14 he said: "Indeed, my position is exactly that of Sutton who argued that it would be purely a matter of chance as to which daughter cell a particular chromosome would enter."

As indicated above, a brief criticism of Voinov's (32) interpretation of the double nucleinic body will be given. If his interpretation were correct that the nucleolus contains all the condensed chromatic matter, he is wrong in claiming that this has been described only in ovogenesis. I would refer him to Blackman's (1 and 2) works on Myriapods.

But he is wrong in saying that the nucleolus and accessory always approach each other in the later growth period. They may be close together in the early part of the growth period, as shown in Fig. 6. The nucleolus may become pale and disappear far removed from the accessory. The latter does not disappear. Voinov says, as Blackman (1) has found in Myriopods, that the nucleolus acts as a reservoir for the chromatin during the rest stage. I doubt this for *Gryllus*, because the nucleolus is not largest when the chromatin is least apparent in the spireme. Voinov (32) was probably misled by his staining results. One lot of my *G. domesticus* shows just such conditions that would lead one to think the chromatin in the growth period is stored in the nucleolus, but all my other lots refute this.

In the "Observation" above I criticised Montgomery's (22) description of synapsis. This is a criticism of the stage as he described it in *Pentatoma* and by implication in *Gryllus*. I do not find a massing as he described it in "Synapsis," but rather a looping as described in "Post-synapsis." This is the stage that Montgomery (25 and 26) has emphasized in his later papers. The ovocytes in the Gryllidæ show the loops crowded to one pole of the nucleus, as Montgomery has described in *Peripatus* and certain salamanders. The loops in the crickets are also present in the reduced number and are probably formed, as Montgomery has suggested, by the union of the parental chromosomes into pairs. He finds that the closer union is at the central pole, but my material shows more free ends at the central pole (see Fig. 36). I should conclude that the closer union is at the distal pole. The spermatocytes are so small that conditions there are much more difficult to decipher. Further study will probably clear up this matter and reveal the method of formation of the various chromosomes in the growth period.

It gives me pleasure to express my gratitude to Dr. Frank R. Lillie for reading this manuscript, as well as much for helpful encouragement during the progress of the work.

Hull Zoölogical Laboratory,
University of Chicago,
August 6, 1904.

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EXPLANATION OF PLATE I.

All drawings were carefully outlined with a camera lucida and the details filled in afterwards. For all the figures except 22 a Leitz $\frac{1}{16}$ objective and a Zeiss ocular 18 was used. The reduction is approximately $\frac{2}{5}$ giving a final magnification of 2,900 diameters.

All the figures are taken from *Gryllus domesticus* except 22 and 24 from *G assimilis* and 36 from *Scapteriscus didactylus.*

The drawings are all numbered as nearly as possible in the order of the stages of development represented. Figs. 18, 19 and 22 are exceptions.

In all figures x = accessory chromosome, n = nucleolus.

FIG. 1. Polar view of equatorial plate of a spermatogonial division. Accessory bent into a U.

FIG. 2. Idem. Accessory stretched out. The numbers indicate the probable pairs of chromosomes.

FIG. 3. Side view of spermatogonial spindle, the chromosomes just separating.

FIG. 4. Early stage of growth period. Accessory adherent to one side. The cell wall as in many other cases is not drawn as a definite line, because it is in many cases not visibly differentiated by the stain and the exact limits between cells can not be made out.

FIG. 5. Later growth period. The chromatin in loops of spireme.

FIGS. 6 AND 7. Later stages of the spireme. The chromatin still more or less in loops. The accessory and nucleolus in juxta-position. Accessory in 6 lies in reality against wall.

FIG. 8. Later than above. One chromosome forms a ring. Accessory and nucleolus at almost opposite sides of the nucleus.

FIG. 9. Fragment of cell showing longitudinally split chromatin rods.

FIG. 10. Chromatin somewhat more diffused. Accessory and nucleolus lie together, but latter very pale in color.

FIG. 11. Late prophase, the accessory dense and with smooth contour. Ordinary chromosomes somewhat granular and with ragged contour. Chromosome 6 was drawn from next section.

FIG. 12. Polar view of very late prophase or metaphase of first spermatocyte spindle. Chromosomes lie at different levels.

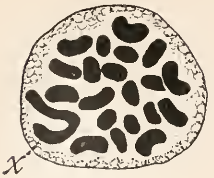

Fig. 1

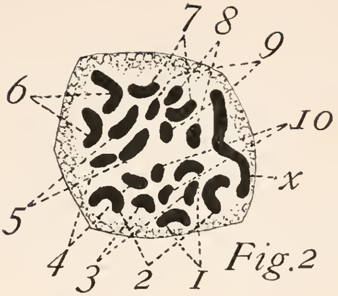
7
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Fig. 2

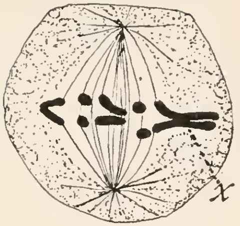


Fig. 3

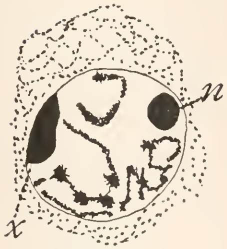


Fig. 4

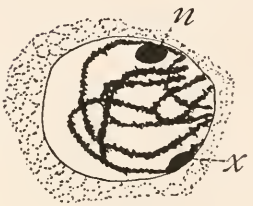


Fig. 5

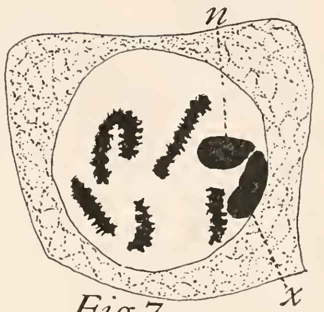


Fig. 7

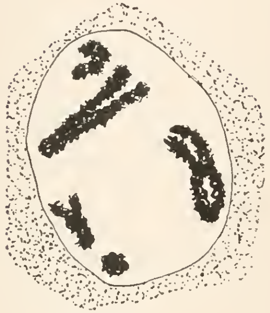


Fig. 9

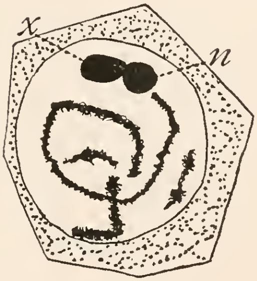


Fig. 6

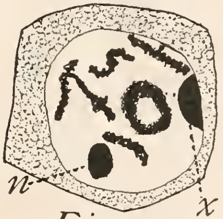


Fig. 8

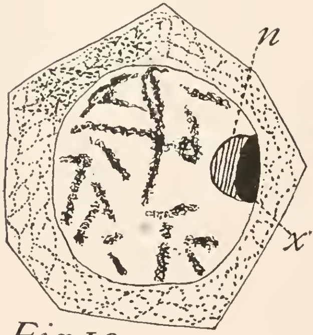


Fig. 10

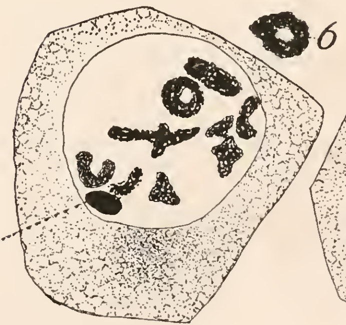


Fig. 11

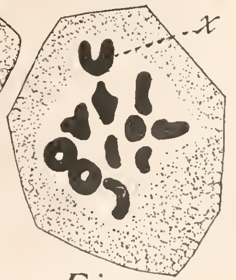


Fig. 12

Explanation of Plate II.

Figs. 13, 14 and 15. Lateral views of the spindle. Chromosomes show various shapes.

Fig. 16. Lateral view of chromosomes showing one ring broken on one side.

Fig. 17. Metaphase showing a ring separating.

Fig. 18. Groups *a*, *b* and *c* show different shapes of chromosomes. Each group was drawn from a single nucleus. Group *a* is from very late prophase; that is, not all the chromosomes have reached the equator of the spindle. The third chromosome in *b* is the accessory.

Fig. 19. A drawing made from a smear preparation showing the various shapes. The chromosome marked *u* has the ends crossed and may be the result of pressure on the nucleus.

Fig. 20. Anaphase showing accessory nearer one pole and outside the spindle.

Fig. 21. Later anaphase, accessory caught in the center and showing constrictions.

Fig. 22. Telophase showing accessory separated by cell walls. Magnification, 1,860 diameters.

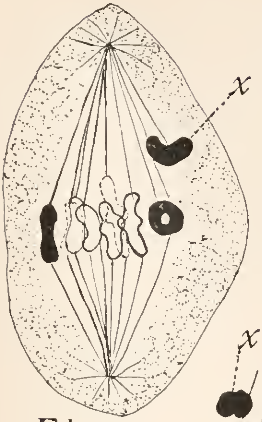
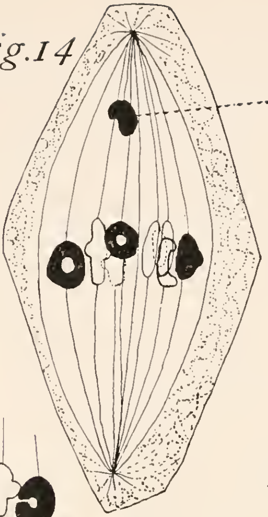


Fig.13

Fig.14



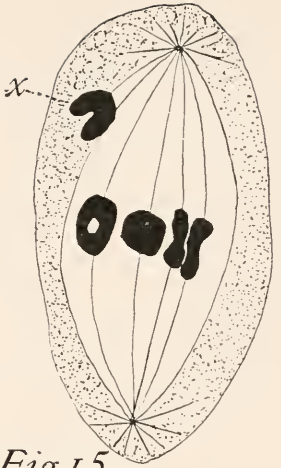


Fig.15

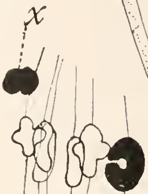


Fig.16

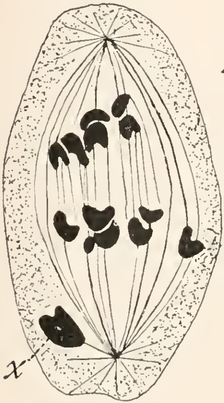


Fig.20

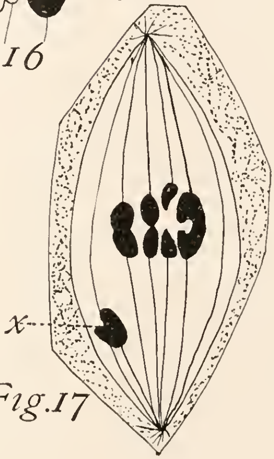


Fig.17

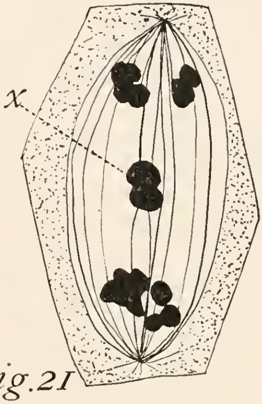


Fig.21

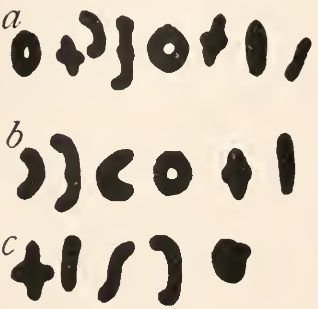


Fig:18

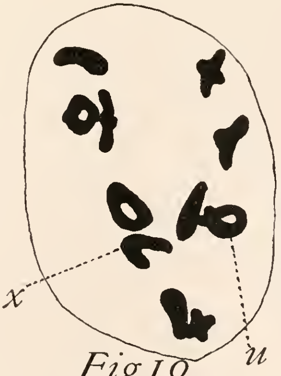


Fig.19

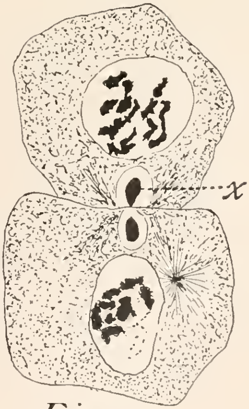


Fig.22

EXPLANATION OF PLATE III.

FIG. 23. Late anaphase. Centrosome divided. Accessory lagging.

FIG. 24. Late anaphase. Spindle between the accessory and the ordinary chromosomes.

FIGS. 25, 26 AND 27 show conditions of the semiresting stage. Accessory has its own vesicle. The ordinary chromosomes are vesicular in 25 and 26.

FIGS. 28 AND 29. Polar views of second spermatocyte spindle. Chromosomes crowded.

FIG. 30. Side view of second spindle.

FIG. 31. Polar view of second spindle. Chromosomes not so crowded.

FIGS. 32 AND 33. Anaphases of second spindle; 32 shows accessory.

FIGS. 34 AND 35. Polar views of anaphases of second spindle. The twin cell of 34 shows the other part of the divided accessory.

FIG. 36. Ovocyte of *Scapteriscus*. Synapsis stage.

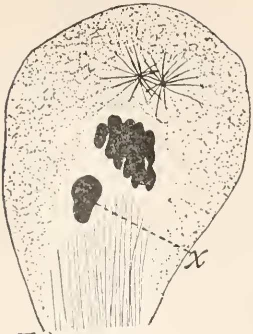


Fig.23

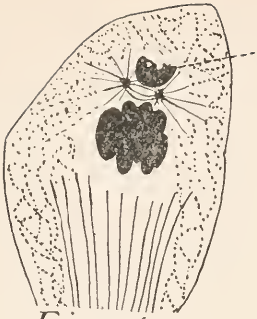


Fig.24

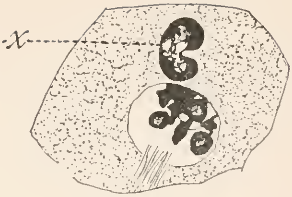


Fig.26

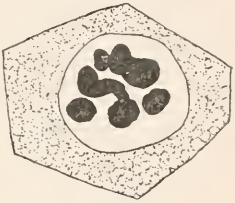


Fig.28

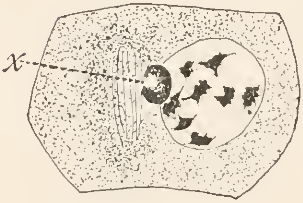


Fig.27

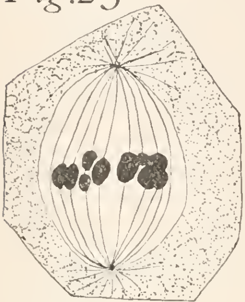


Fig.30

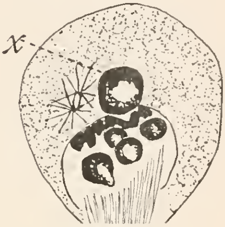


Fig.25

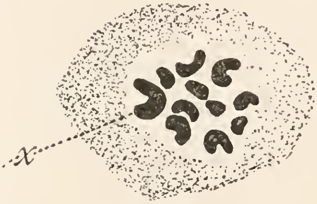


Fig.31

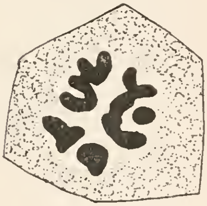


Fig.29

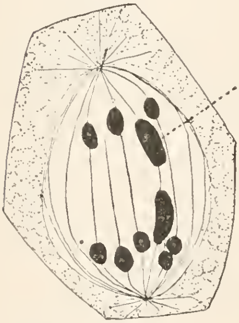


Fig.32

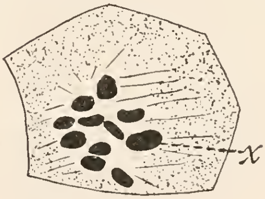


Fig.34

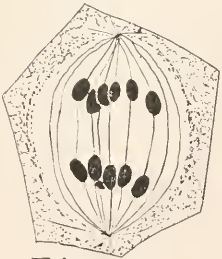


Fig.33

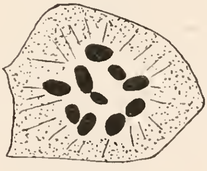


Fig.35

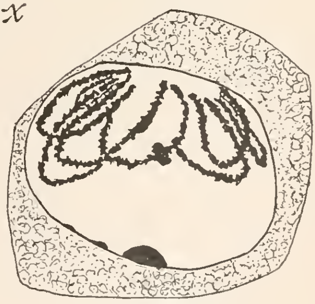


Fig.36

THE PHYLOGENY OF THE TERMITES.

WILLIAM MORTON WHEELER.

Among the changes that have been suggested within recent years in the classification of the larger groups of insects, none are more important than those affecting certain groups isolated by Burmeister ('39) and Brauer ('85) under the designation Corrodentia from the miscellaneous complex variously known to the older entomologists as Orthoptera, Neuroptera and Pseudoneuroptera. For Burmeister the Corrodentia embraced the Termitidæ ("white ants"), the Embiidæ and the Psocidæ ("book-lice"). Brauer included under the Corrodentia the Termitidæ, the Psocidæ and the Mallophaga ("bird-lice"), but assigned the Embiidæ to the Orthoptera with a query. More recently some authors have been inclined to combine the classifications of Burmeister and Brauer. Enderlein ('03), for example, divides the order Corrodentia into three suborders, one comprising only the Psocidæ and called by him Copegnatha, another comprising the Mallophaga, and a third (Isoptera), sharply separated from the two others and comprising the Termitidæ and the Embiidæ.

Börner ('04) has raised Enderlein's suborder Isoptera to ordinal rank and thus removed the termites and Embiids from the order Corrodentia, in which he leaves only the Psocidæ and the Mallophaga.

Handlirsch, in two valuable and suggestive papers on the classification of insects ('03 and '04), maintains that the Corrodentia (in the sense of Enderlein) must be resolved into four different orders: the Isoptera, in Comstock's sense ('99, pp. 96–97),[1] and including the termites only, the Psocidæ, to which Handlirsch limits the term Corrodentia, the Mallophaga (Nitsch) and the Embiaria (Handlirsch). Far from regarding the Embiids as related to the termites, he places these groups in different subclasses (Blattæformia and Embioidea). Thus the old group Corrodentia has undergone a complete taxonomic disintegration, and it would probably be best to abandon the term altogether and not limit it with Handlirsch to the Psocidæ.

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[1] I infer from Hagen's monograph of the termites ('58), that the term Isoptera goes back to Brullé and was by him used in the sense of Termitidæ. If this is true the term and conception should not be attributed to Comstock.

Shipley ('04), in a paper devoted to the emendation of the names of the insect orders, follows Sharp, who seems to agree with Handlirsch in recognizing four independent orders : Isoptera (Termitidæ), Embioptera (Embiidæ), Psocoptera (Psocidæ) and Lipoptera (Mallophaga). Three of these four ordinal names have been coined by Shipley for the sake of making all the insect orders end in *ptera*, after the well-known classical models. Both Handlirsch and Borner ('04) repudiate this attempt of Shipley, the former on the grounds of priority, the latter because some of these names, like Psocoptera and Embioptera are by no means homogeneous in their formation with the classic examples Lepidoptera, Coleoptera, Neuroptera, etc. It seems to me that if the law of priority in nomenclature is to mean anything, it must be applied to the names of the larger groups as rigorously as to the names of genera and species.

Concerning the termites, with which I am more concerned in this paper, Handlirsch ('04, p. 738) makes the following statement : ". . . they have been regarded as having a very primitive organization, and the circumstance that many paleozoic and mesozoic forms have been (erroneously) claimed to be termites, seemed to support this view. Among other characters the homonomy of the wings has been interpreted as primitive. A study of these organs, however, shows that they are highly specialized and that the homonomy has come about through atrophy of the anal border of the fore and hind wing. The termite wing is a Blattid wing with strongly reduced anal area, so that the homonomy is a secondary condition. Quite as highly modified are the termites in respect to their polymorphism, the formation of societies, the reduction of the cerci, the multiplication and lengthening of the ovarioles, the reduction in the number of the Malpighian vessels, etc. The feebler concentration of the thoracic segments seems to have accompanied the specialization of the wings or rather the decrease in their function, and is, at all events, to be regarded as no more primitive than the similar condition in the fleas. It is quite as impossible to derive the termites from the Embiidæ as the Embiids from the termites. The wings of the Embiids are specialized in an entirely different direction, the legs are more highly developed, as are also the abdominal appendages, the ocelli have disappeared, etc. With respect to their entire organization the termites may be derived without difficulty from the Blattids, which agree with them also in the position of the legs and in having large coxæ. Interesting is the existence of termite larvæ with prothoracic dilatations, which have no particular function and ultimately disappear."

In this paragraph Handlirsch clearly formulates a view which seems to have been gaining ground ever since Hagen, nearly half a century ago ('58, p. 31) insisted on the close affinity of the Termitidæ with the Blattidæ, or cockroaches.

At this point the subject has been taken up in an interesting paper by Desneux ('04), who has actually found in a North Australian termite (*Mastotermes darwiniensis* Froggatt) a form, which, though a true termite, is nevertheless structurally transitional between the Blattidæ and the other termites. This form, of which only the imago is known, is regarded by Desneux as representing a new subfamily (Mastotermitinæ) and leads him to propose the following arrangement of the Isoptera, or Termitidæ :

- Isoptera, or Termitidæ
  - Termitinæ
    - Termitini (*Termes*).
    - Rhinotermitini (*Rhinotermes* + *Arrhinotermes*).
  - Calotermitinæ
    - Calotermitini
      - Calotermes.
    - Hodotermitini
      - *Hodotermes* + *Podotermes* + *Stolotermes*.
    - *Termopsis*.
  - Mastotermitinæ
    - *Mastotermes*.

The blattoid characters of Mastotermes, as determined by Desneux, are the following : The wings have the typical blattoid neuration, both pairs have a distinct anal area, small and bounded by an arched groove in the fore wing, but in the hind wing highly developed and folded during repose. In the anterior wing the basal corneous portion is considerable. The neuration of the hind wing is very different from that of the fore wing, and the anal lobe is clearly marked off by a deep notch. The head is without a fontanelle, the antennæ are long and 30-jointed, the labrum does not project, the prothorax is large, larger, in fact, than the head, the tibiæ have a row of spines along their sides and four spines at the apex. The tarsi are 5-jointed and furnished with a small pulvillus.

Desneux's study of Mastotermes thus supplies the keystone to a taxonomic arch which students of the lower insects have been building for some time past. The structure is, however, still incomplete, since only the facts of comparative anatomy seem to have been consulted, and due regard for the facts of paleontology, geographical distribution, embryology and ethology must be had before we can accept the derivation of the Termitidæ from the Blattidæ as established beyond contradiction. As Handlirsch has studied fossil insects extensively, it is probable that he has at least found nothing in paleontology to contradict his theory. In regard to geographical distribution there is no difficulty, but instead a singular resemblance between the two groups of insects in question, inasmuch as both of them are cosmopolitan and, with the exception of a very few species, confined to tropical and subtropical countries. In other words, countries which are most favorable to the development of Blattidæ also have a rich termite fauna. But how stands it with the embryology and ethology of the Blattidæ and Termitidæ? I believe that the views of Handlirsch and Desneux are not without some support from these sources also, as I shall attempt to show in the following paragraphs.

A number of investigators have studied the development of various Orthoptera, including the Blattidæ, and Knower ('00) has furnished us with a valuable paper on the embryology of a termite (*Eutermes*). In comparing the development of these insects with one another we are struck with the remarkable similarity between the termite and the Orthoptera, especially certain Saltatoria (Acridiidæ and Gryllidæ). This resemblance, which was recognized by Knower, is seen in the simple nature of the chorion, the absence of a vitelline membrane, the structure and consistency of the yolk, the great volume of the yolk as compared with that of the incipient germ-band, the position of the latter on the posterior ventral surface of the egg, the pronounced anatreptic and katatreptic movements of the embryo, etc. Comparison of the termite with the Blattid shows great similarity in the structure of the micropyles, but, as I have pointed out ('89, '93), the blastokinetic, or embryonic movements in the latter insect are very feeble, not sufficient, in fact, to carry the embryo from the anterior to the posterior surface of the yolk. This slight movement I interpreted as a vestige of the more pronounced blastokinesis of the Saltatoria. If this view is correct, we must suppose the Termitidæ to have retained in the more pronounced movements of the germ-band somewhat more primitive orthopteroid conditions than the Gressoria (Blattidæ and Mantidæ). This is, of course, not necessarily fatal to a derivation of the termites from the Blattidæ, since a modification of the embryonic development in the direction of a partial suppression of blastokinesis may have supervened within the Blattid group after the Termitidæ had diverged from the more primitive family stock.[1]

At first blush there would seem to be no ethological relationship between the Blattidæ and the Termitidæ. The former are regarded as omnivorous insects without a social organization whereas the latter have a specialized diet of cellulose and present a social organization only equalled or surpassed in complexity by that of the ants, certain wasps and bees. Somewhat closer

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[1] Knower seems to have misunderstood my position in regard to the superficial and immersed germ-bands of insects. At any rate his long discussion of this and kindred matters tends only to befog the whole subject. I expressly maintained ('93, p. 68) that the stationary, superficial germ-band is primitive in the Arthropoda in general, and probably also in the insects in particular, but that in the latter class blastokinesis, with or without immersion, early established itself since it is found in many primitive orders. Later these movements were abolished (the Blattidæ, *e. g.*, show them very feebly), so that the higher insects (Coleoptera, Hymenoptera, Diptera, etc.) have secondarily returned to the primitive type of an almost stationary, superficial germ-band. I believe the chief stress in these considerations is to be laid on the movements of the embryo and not on the more incidental superficiality or immersion of the embryo. Whether embryos like those in Knower's stages *M* and *N* (Pl. 33) are to be regarded as superficial or immersed is open to serious doubt. A form like the locustid *Xiphidium* is immersed during anatrepsis, but superficial during katatrepsis. In passing I may express my inability to conceive why Knower courts confusion by inverting all the figures of his termite embryos.

study, however, shows, unmistakable ethological similarities between the two families. Both are strongly heliophobic, or dark-loving (negatively heliotactic) animals and hence also positively contact-loving or thigmotactic. Our wild cockroaches, like the termites live in dead or decomposing wood or in the soil. Furthermore, it can be shown that there are adumbrations of social life among the Blattidæ. Our domestic species are somewhat gregarious in their adolescent and adult stages. Then, too, the mother cockroach deposits her eggs in a peculiar oötheca which she carries about in a kind of brood chamber formed by an infolding of the terminal segments of the abdomen. The oötheca is, however, deposited before the young hatch. Entomological treatises repeat the statement that the female "croton bug" (*Phyllodromia germanica*) assists the young in escaping from the oötheca. This observation is traceable to Hummel ('29) who recorded it in a work which has become rather rare. The pertinent passage may, however, be found quoted in full in the works of Audouin and Brullé ('35, pp. 36, 37) and Serville ('39, pp. 59, 60). The fact that in some of our species of Blattidæ the young can escape from the oötheca without maternal assistance, casts doubt on these old observations. The female in these species exhibits only the first rudiments of a social instinct in the care of the young till they are nearly ready to hatch.[1]

A further extension of maternal care is seen in the beautiful green South American cockroach, *Panchlora viridis*, which is sometimes imported alive into stores and houses in New York and Brooklyn. Riley, in three short papers ('90, '91*a*, '91*b*), claimed that this insect is viviparous, but a moment's examination of the very facts he has recorded, shows that it cannot even be regarded as ovoviviparous. In his third paper ('91*b*) he figures the semi-circular egg-mass of the insect with two rows of embryos arranged with their ventral surfaces face to face, just as others and myself have shown them to be arranged in the oötheca of *Phyllodromia* and *Periplaneta*. Moreover, a delicate membrane, which Riley himself interprets as a vestigial oöthecal envelope, encloses at least the posterior half of the eggs. Now this egg-mass, which is obviously only a slightly modified oötheca, is, according to Riley's statement, enclosed, not in the reproductive organs proper or body cavity of the mother insect, but in the oöthecal chamber. But this chamber, which also embraces at least one end of the oötheca in other Blattidæ, is morphologically *outside* of the body. Hence there can be no viviparity or even ovoviviparity in this case. *Panchlora* simply retains the oötheca completely within the oöthecal chamber till the young are ready to hatch.[1] This Blattid therefore represents a further stage in the care of the brood than is to be found in our domestic cockroaches.[2]

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[1] That the maternal instincts of the "croton bug" are highly variable is proved by an observation communicated to me by Mr. Wm. Beutenmüller while these pages are going through the press. He found one of these insects surrounded by her young and still retaining in her oöthecal chamber the empty oötheca from which they had just escaped.

A more advanced, though still very crude, stage of social organization seems to be represented in certain Blattidæ like the singular North American *Dasyposoma* (*Cryptocercus*) *punctulata* Scudder ('62, pp. 419–421). This insect, which has been recorded from Virginia, North Carolina, Pennsylvania and New York, appears to be a very primitive form. v. Wattenwyl ('93), Scudder ('97, pp. 13, 15) and de Saussure ('95*b*) regard it as belonging to the subfamily Panesthinæ, a group comprising a number of Australian and Oriental species.[3] Comparison of a fine series of this insect collected by Mr. Wm. Beutenmüller in the mountains of North Carolina, with a series of the Australian *Panesthia cribrata* Saussure in the Edwards Collection of the American Museum of Natural History shows many striking resemblances in shape, color, punctulation, etc. Both sexes of the American species, however, are apterous. Mr. Beutenmüller has given me some notes on the habits of this insect. He found it living in colonies of fifty or more individuals, old and young together, burrowing in stumps, most frequently of oak trees, that had nearly or quite reached the red stage of ligneous decay. The burrows were sharply defined and close together and reminded him of the burrows of the beetle *Passalus cornutus*. Here we have a resemblance to the termites not only in the rude social life but also in the wood-boring habit.

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[1] This interpretation of Riley's work is confirmed by an examination of two dried female specimens of *Panchlora viridis* in the collections of the American Museum of Natural History. Each showed a shrivelled oötheca in the oöthecal chamber. On dissecting one of the specimens I found the oötheca to be of large size and bent in the form of a *C*, with its two ends almost meeting. It contained seventy-two embryos arranged in two series as in the common cockroaches.

[2] Among primitive insects the Forficulidæ, or earwigs, have long been known to care for their eggs, collecting them when dispersed and, according to some observers, even carrying them to places of more favorable temperature and moisture. Similar habits must be very ancient among the air-breathing arthropods as we find them well developed in certain Myriopods (*Geophilus*), which not only brood over their eggs but guard their young after hatching.

[3] De Saussure und Zehntner ('95*a*) however, include *Cryptocercus* in another primitive subfamily, the Perisphæriinæ.

Further observations on *Dasyposoma* and other Blattidæ are much needed and may perhaps enable us completely to bridge the gap that separates the rudimental social instincts of such insects from the complex social instincts of the Termitidæ. The recent revival in the study of termites certainly calls for a renewed interest in the habits of the much neglected Blattidæ.

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ON THE ANATOMY AND EMBRYOLOGY OF THE NERVOUS SYSTEM OF THE SCORPION.[1]

J. F. McCLENDON.

Early in May, 1903, at the suggestion of Dr. William Morton Wheeler, at that time professor of zoölogy in the University of Texas, I began collecting females and preserving embryos of the scorpion here considered. I found the scorpions under stones on certain hills covered with scrub oak, scrub cedar or grass in the neighborhood of Austin, Texas. They prefer dry areas with an abundance of broad, flat stones, or at least stones lying loosely on the ground, under which they hide. The first scorpions collected (May 13) contained eggs in early cleavage stages, and the last scorpions taken that year, June 10, contained embryos with pigment well developed in the eyes. The next year the scorpions developed earlier, and by the first of June I procured successive stages of the embryo up to the time of birth. Most of the work was done at the University of Texas during the session of 1903–04, under the direction of Dr. Thomas H. Montgomery, professor of zoölogy.

Material and Methods.

The species of scorpion I worked on is that described and figured by R. J. Pocock in the Biologia Centrali Americana under the name of *Centrurioides vittatus* Say. I sent some specimens to Professor Kraepelin, and he identified them as *Centrurus infamatus* C. L. Koch. This is probably synonymous with *Scorpio carolinianus* Palisot de Beauvois, and probably the species Patten (1890) worked on, *Buthus carolinianus*, as stated in his paper.

The embryos, either in the ovarian tubules or dissected out, were fixed in Lee's picro-acetic, Kleinenberg's picro-sulphuric, or Tower's alcohol-corrosive-aceto-nitric (*Zoöl. Jahr. Anat. Ontog. d. Thiere*, Vol. 17, heft 3, 1903). As all the embryos taken from one mother are in the same stage of development it is convenient to keep them in a separate vial. The embryos may be studied in alcohol, but for cleared preparations the following process was found to give good results: The yolk was removed with needles and fine brushes and the embryos were then stained in Delafield's hæmatoxylin, diluted with water acidulated with a trace of picro-sulphuric, dehydrated with alcohols acidulated with the same and mounted in balsam. The intensity of the stain must be controlled by the proportions of the stain and acid used, and the time they are allowed to act. Eggs for sectioning were imbedded in paraffin and the block cut so as to remove as much of the yolk as possible without injury to the parts desired, then reimbedded and sectioned and stained in Heidenhain's iron-hæmatoxylin. It is difficult, even with the use of the mastic-collodion process, to obtain perfect series of the entire egg. Embryos ready to hatch could be to some extent dissected, but contained a large amount of yolk.

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[1] Contribution from the Zoölogical Laboratory of the University of Texas, No. 60.

Adults for dissection were opened while fresh and the blood washed out and liver partly removed in physiological salt solution, then hardened in weak alcohol. Adults for sectioning were taken immediately after moulting and injected with Flemming's fluid, in which they were left six days, followed by pyroligneous acid (v. Mährenthal), or pyrogallic acid (Hermann) for two or three days; or they were stained with Heidenhain's iron hæmatoxylin after any good fixative. This latter process brings out the nerve-fiber tracts in the fibrous substance of the nerve center. In staining on the slide, parts of sections were often washed off, though they had been attached with Meyer's albumen fixative, flattened with the aid of warm water, and dried for twenty-four hours. To prevent this I took the slides after drying and painted them with three fourths per cent. celloidin (Gage) and

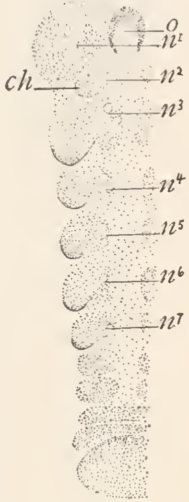


FIG. 1. Camera lucida drawing of the right side of a cleared surface preparation of the embryo of *Centrurioides vittatus* as early as the neuromeres are clearly distinguishable, × 45. *ch*, chelicera; n^{1-7}, first to seventh neuromeres; *o*, mouth.

dried them again. After this treatment no sections came off unless they were left too long in absolute alcohol.

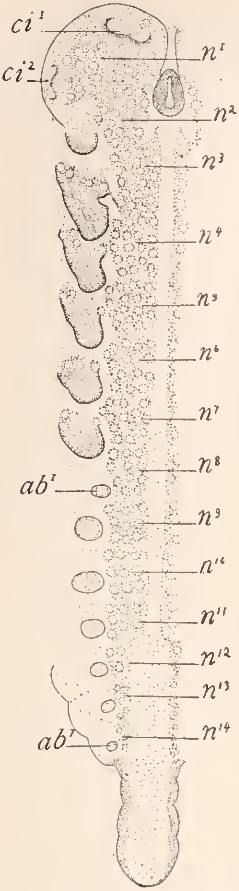


FIG. 2. Second stage of same, × 45. The post-abdomen is turned under to show the posterior neuromeres. ab^{1-7}, first to seventh abdominal appendages; ci^1, first cerebral invagination, to join later with its fellow of the opposite side to form the semi-lunar lobe; ci^2, second cerebral invagination; n^{1-14}, first to fourteenth neuromeres.

HISTORICAL.

As early as 1870, Metschnikoff represented the neuromeres as paired thickenings of the ectoderm on surface views of the scorpion embryo, there being one neuromere for the segment bearing the eyes, one for the segment bearing the chelicera and so on for succeeding segments back to the point where the fold of the post-abdomen hid them from view. A "longitudinal furrow" runs from the mouth backward, separating the two halves of each neuromere. His figures show also the relation of the anterior neuromeres to the mouth — that is to say the mouth is formed in the first segment and during development moves to the posterior part of the second segment, by which process the second neuromere becomes pre-oral.

Kowalevsky and Schulgin (1886) described the transformation of undifferentiated ectoderm into nervous tissue. The ectoderm, in the regions of the future ganglia, begins to thicken and the cells to increase in number rapidly and minute pits are formed all over its free surface. These pits are gradually filled up by the growth of the cells forming their walls. This method of cell increase and growth was supposed by the writers to possess peculiar advantages. Unfortunately they gave no figures.

Saint-Remy made a comparative study of the "brains" of different groups of air-breathing arthropods, which he published in several papers, the last of which (1887) contains the results of all his work on the subject. He worked out the nerve cell-groups and the nerve fiber-tracts and came to the conclusion that there are *two* pre-cheliceral neuromeres in the scorpion. But his observations do not seem to support his conclusions, for, while he showed that there are, corresponding to these two neuromeres, two pairs of optic lobes, he described their nerve-fiber tracts communicating with the interior of the "brain" as united to form one. He showed great complexity in the structure of the "brain."

In 1890, Patten, in a preliminary paper on the origin of vertebrates from arachnids, compared the arachnid cephalothoracic nerve mass, or "brain," with the vertebrate brain, turning the former up-side-down in order to get the proper relation with the digestive tube. In the scorpion embryo he found three precheliceral neuromeres, each with a pair of optic ganglia. In each optic ganglion was an invagination. The cephalo-thoracic nerve mass was composed of thirteen neuromeres. In the adult, a typical neuromere had a pair of "neural" nerves and two

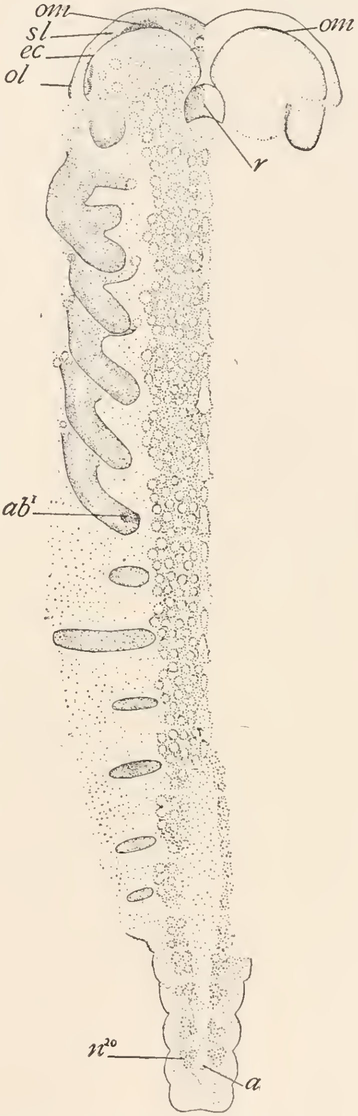


FIG. 3. Third stage of same, × 45. On one side the head is represented as an opaque object. *a*, anus; ab^1, first abdominal appendage; *ec*, anterior margin of the ectoderm growing over the first neuromere; n^{20}, twentieth neuromere; *ol*, ectodermal thickening to form the lateral eye; *om*, ectodermal invagination to form the median eye sac; *sl*, semilunar lobe; *r*, rostrum.

pairs of "hæmal" nerves, and each neural nerve had a ganglion at its base. The first neuromere was nerveless; the second had a pair of neural nerves to the median eyes and a pair of hæmal nerves to muscles; the third neuromere had a pair of neural nerves to the lateral eyes and two pairs of hæmal nerves to muscles; neuromeres four to nine had each a pair of neural nerves to appendages and two pairs of hæmal nerves to internal muscles. The last four neuromeres had their neural nerves all united into one pair, that innervated the pectines, but each had two pairs of hæmal nerves which went to make up a system homologous to the vagus nerve of vertebrates. In each of the neuromeres succeeding the "brain" there was one pair of neural nerves, and only one pair of hæmal nerves well developed. These two pairs of nerves united a short distance from the nerve center to form a single pair homologous to spinal nerves of vertebrates. Patten figured the pits that form in the neuromeres in the early embryonic stages, and he supposed that they represented sense-organs from which the nervous system arose. In the course of development a piece is constricted off of each ganglion of the ventral chain in each segment and added to the succeeding ganglion.

Viallanes' work (1893, 2) was chiefly on *Limulus* and does not bear directly on our subject, but it is interesting to note that he held the view that there is only one pre-cheliceral neuromere in the arachnid type of nervous system.

Braner (1894–5) worked out the earlier half of the embryology of the scorpion. He clearly described and figured the early development of the eyes and their relation to the nervous system, a matter that had been confused by all previous workers on the subject. He supported Kowalevsky and Schulgin's explanation of the pits in the embryonic nerve tissue, rather than Patten's. The ectoderm was found to grow over the ventral chain from the sides. Brauer reckoned the number of neuromeres by the cross-commissures, there being two in front of the cheliceral segment. But it is not evident why he should count cross-commissures rather than nerves or ganglia, which are equally characteristic parts of a neuromere. Such a criterion for neuromere would hardly be accepted in a form like *Peripatus*.

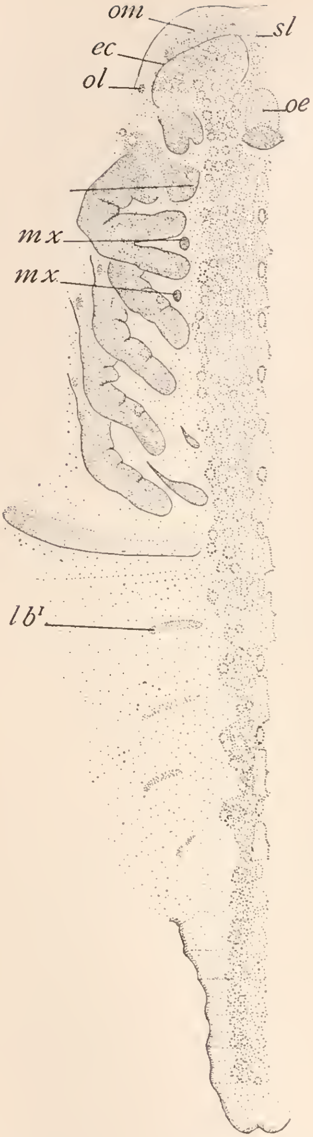
Laurie (1896, 1 and 2) described the variation in positions of the abdominal ganglia in different species of scorpion.

Police described the anatomy of the nervous system of the scorpion (1901, 1) and the histology of the subintestinal portion (1901, 2). He described the typical neuromere as a pair of ganglia fused in the mid-line and giving off two pairs of nerves, "external" and "internal." The longitudinal connectives continue through the fibrous substance of the nerve center as a pair of "central columns," which are connected by two tracts of commissural fibers, anterior and posterior. There is also a pair of "ventral columns." The central columns send nerve fibers into both pairs of nerves, those going into the internal nerves leaving the columns at the posterior cross-commissure. The ventral columns send nerve fibers into the external nerves. This classification of the nerves as "external" and "internal" is unfortunate, for, although in some abdominal neuromeres the external nerves do arise from a broader part of the nerve center than the internal, and are properly described by the name, in the thoracic neuromeres they do not. They had better be described as anterior and posterior, since during the early embryology they all conform to this description.

Lankester (1904) held the view that there is only one precheliceral neuromere.

Descriptive.

Stage 1.—(Fig. 1.) This is the earliest stage in which the neuromeres can be clearly distinguished from the rudiments of the appendages. In addition to the telson, which is not considered as a segment in the strict sense of the term, eleven segments have been formed. Appendages are appearing on segments two to seven. Neuromeres can be distinguished on segments one to seven, being represented by paired thickenings of the ectoderm indented with small pits. The first neuromere, composed of a pair of "cephalic lobes," is much larger than any of the others. The oral invagination (*o*) is appearing in the first segment. From the mouth backward to the tenth segment in this stage is a median depression, the longitudinal furrow of Metschnikoff or median furrow of Patten. The depression is caused by the ectoderm being thinner in this region.



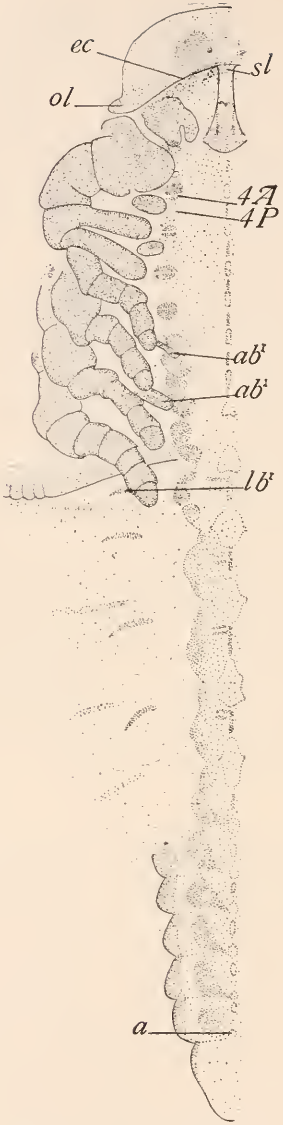
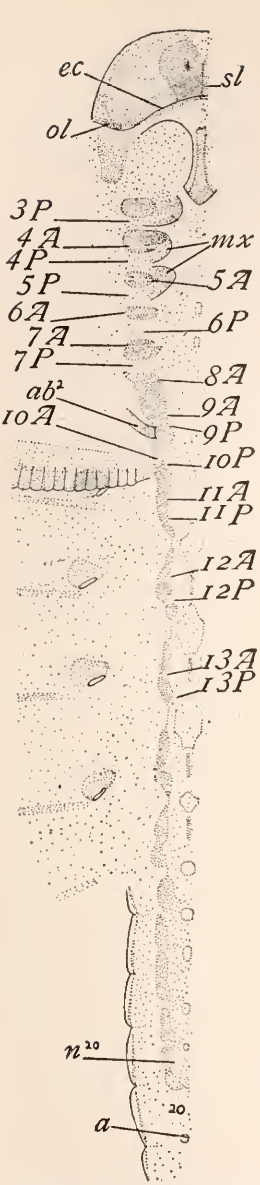


FIG. 4. Fourth stage of same, × 45. *ec*, anterior margin of ectoderm growing over the first neuromere; lb^1, first lung book; *mx*, maxillaria; *œ*, œsophagus; *ol*, latera-eye; *om*, median eye sac; *sl*, semilunar lobe.

FIG. 5. Fifth stage of same, × 45. *a*, anus; ab^{1-2}, first and second abdominal appendages; *ec*, anterior border of ectoderm growing over first neuromere; lb' first, lung book; *ol*, lateral eye; *sl*, semilunar lobe; 4*A*, anterior nerve of the fourth neuromere 4*P*, posterior nerve of fourth neuromere.

Stage 2. — (Fig. 2.) Seventeen segments have formed. Appendages are present on segments two to fourteen and neuromeres in segments one to fourteen. The median furrow is continued backward and is very broad in segments twelve to fourteen, a condition probably due to the pressure of the post abdomen, which is turned under the pre-abdomen during the development of the embryo and not straightened out as shown in the figure. In addition to the numerous pits two pairs of invaginations have appeared in the first neuromere (ci^1, ci^2). These invaginations, while larger than the pits, are probably similar to them physiologically, for like them they are only temporary structures, being finally filled up by the growth of the cells forming their walls. Pits like those in the neuromeres have formed, externally and internally at the bases of the thoracic appendages and probably represent sense organs, as Patten maintains. It is not probable however that the pits in the neuromeres represent sense organs, since the embryology of the scorpion is of so specialized a type that we would not expect to find such remotely ancestral structures repeated here, when not repeated in more generalized types. The mouth has begun to move backward. This is accomplished in two ways : First there is an actual displacement, the ectoderm surrounding the mouth being thin and probably offering little resistance ; second, the mouth opening elongates and the anterior portion closes by the growing together and fusing of the two sides, the remaining opening being further back than the center of the original opening. The closure of the anterior portion of the mouth forms a lip called the rostrum (Fig. 3, *r*). It is thought by some that the rostrum represents the fusion of a pair of appendages. That it is formed by *fusion* is clear, but the only evidence I see in favor of regarding it as derived from *appendages* is the fact that in the later embryo and the adult it is innervated by a special nerve (Fig. 8, *R*). This nerve innervates the muscles of the anterior part of the œsophagus, or pharynx, also and might as well be called a pharyngeal as a rostral nerve.

Stage 3. — (Fig. 3.) Twenty segments, the complete number, have formed, and neuromeres are present in all of them. In the anterior part of each of segments three to twelve an elliptical


ec
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7P
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ab²
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9P
10P
11A
11P
12A
12P
13A
13P
n²⁰
20
a

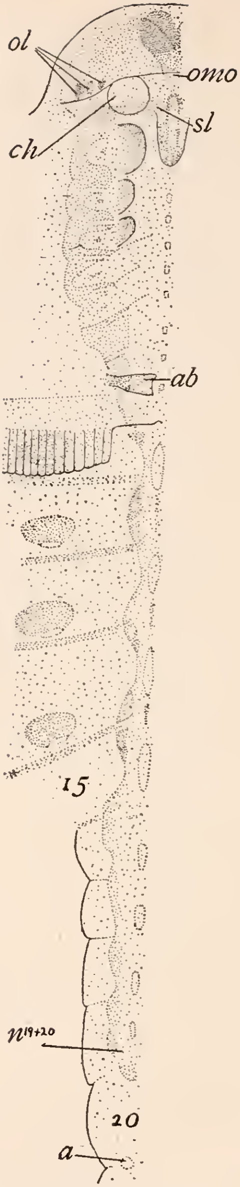

ol
omo
sl
ch
ab
15
n19+20
20
a

FIG. 6. Sixth stage of same, × 45. The cephalo-thoracic appendages have been removed. Some of the details have been filled in from a study of serial sections. *a*, anus; ab^2, second abdominal appendage; *ec*, anterior border of ectoderm growing over first neuromere; *mx*, maxillaria; n^{20}, twentieth neuromere; *ol*, lateral eye; *sl*, semilunar lobe; 4–13*A*, anterior nerves of fourth to thirteenth neuromeres; 3–7, 9–13*P*, posterior nerves of corresponding neuromeres; 20, twentieth body segment.

FIG. 7. Seventh stage of same, × 45. Cephalothoracic appendages removed; *a*, anus; ab^2, second abdominal appendage; ch^1, articulation of the chelicera; n^{19+2}, fusion of the nineteenth and twentieth nerve centers; *ol*, lateral eye; *omo*, common neck of the two median eye sacs; 15–20, fifteenth to twentieth body segments.

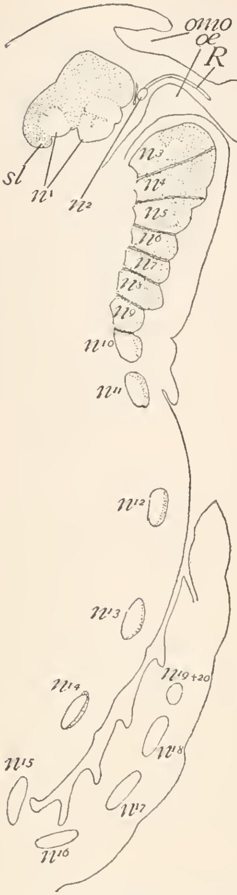
invagination is forming in the median furrow. The tissue thus invaginated is destined to form nerve tissue connecting the two halves of the neuromere in each of the segments mentioned. The process is carried backward to the posterior segments in later stages (Figs. 4, 5). These invaginations have the same appearance as the numerous pits so characteristic of the nerve tissue, differing from the latter however in time of appearance and in shape. The elongated shape is probably due to the narrowness of the median furrow. More pits are constantly being added to the neuromeres, and the late appearance of those in the median furrow is more apparent than real. In no case have I observed the invaginations of the median furrow to coalesce and form a temporary central canal, as Patten claims to have seen. The walls of the first pair of invaginations in the first neuromere have thickened and are fusing in the mid-line to form the semi-lunar-lobe of Patten (*sl*). The ectoderm has begun to grow backward over the first neuromere, its free border being shown at *ec*. On each side there is an invagination in the ectoderm close to the free border, forming the median-eye-sac (*om*), and an ectodermal thickening, to form the lateral eyes (*ol*). The ectoderm has begun to grow over the ventral chain from the sides, but this is not shown in the figure. Patten describes three pairs of ganglionic invaginations in the "cephalic lobes" the first of which I have represented in Fig. 2, ci^1, and Fig. 3, *sl*. The second, he describes in relation to the median eyes. I was unable to find it, either in sections or surface views. It is most probable that he referred to the ectodermal invagination or median eye sac (Fig. 3, *om*). The third he describes in relation to the lateral eyes and I have represented it in Fig. 2, ci^2; it may be seen also in Fig. 3 near the lateral eye rudiment (*ol*).

Stage 4.—(Fig. 4.) A glance at Fig. 4 will show that a piece is being constricted off of the posterior portion of each ganglion in segments eleven to fourteen and it will be seen in Fig. 5 that when the neuromeres begin to separate they do so at this newly formed constriction. In other words the posterior portion of one neuromere is constricted off and added to the succeeding neuromere. Patten supposed the neuromeres to be double, and used this fact, of which he was the first observer, to support his view, comparing the process to a re-arrangement of pairs of neuromeres. But the parts that are displaced in the process are small masses of nerve tissue without nerves or cross-commissures and could not be regarded as neuromeres unless associated with separate segments of the body. This, Patten attempted to do, supposing the segments of all arthropods to be double, as shown by the frequent presence of "bifurcated appendages" and the "frequent occurrence of insect monsters having double pairs of legs." On the contrary my observations and reading lead me to believe that, with the exception of the diplopods (*Julus*), it is probable that the segments of all arthropods are single. In the scorpion embryo each segment except the first has one pair of mesoblastic somites and one pair of appendages.

The semilunar lobe (*sl*, indicated by a transverse band shaded slightly darker) has elongated somewhat and has moved upward and backward with the flexure of the anterior part of the neural band. In Figs. 5, 6, 7, 8 and 9 this process may be seen to continue until the anterior part of the nerve band is first perpendicular to the posterior or subenteric portion, and then by continued flexure bends backward. The ectoderm continues to grow over the first neuromere (*ec*). The mouth has continued to move backward, bending the œsophagus in the form of an arc (*æ*), compare Fig. 8, *æ*.

The appendages of the tenth segment are elongating to form the pectines, while those of segments eleven to fourteen have disappeared, their place being taken by lung books. Sterno-coxal processes, called maxillaria by Patten, have appeared on the fourth and fifth segments (*mx*).

Stage 5.—(Fig. 5.) In this stage the pits have almost entirely disappeared from the nervous tissue, being filled up by the growth of the cells composing their walls. The ectoderm has grown over the ventral chain, which has now become more compact. The nerves have begun to assume their definite form (4*A*, 4*P*), that is to say the peripheral nerve fibers are being compacted into nerves that can be distinguished in sections and sometimes in surface views. The ectoderm has grown further back over the head (*ec*). Pigment is beginning to appear in the median eyes.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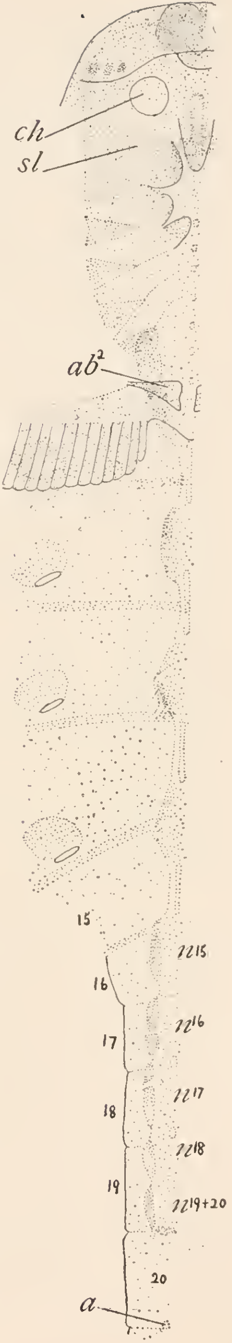
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20
a

FIG. 8. Median sagittal section of an embryo of the seventh stage, × 45 ; *æ*, œsophagus ; *omo*, neck of median eye sacs ; n^{1-20}, cross commissures of first to twentieth neuromeres ; *r*, rostrum ; *R*, rostral nerve ; *sl*, semilunar lobe.

FIG. 9. Eighth stage of same, × 40 ; *a*, anus ; ab^2, second abdominal appendage ; *ch*, cheliceral articulation ; n^{15-20}, fifteenth to twentieth neuromeres ; *sl*, semilunar lobe ; **15–20**, fifteenth to twentieth body segments.

Stage 6. — (Fig. 6.) The median-eye sacs have come together in the mid-line. In this stage I have worked out the nerves of the first thirteen neuromeres. Typically there are two pairs of nerves to each neuromere : an anterior pair ("hæmal" of Patten, "external" of Police) and a posterior pair ("neural" of Patten, "internal" of Police). In Fig. 6 the anterior nerves are shown in neuromeres four to thirteen (4–13, *A*) and the posterior nerves in neuromeres three to seven and nine to thirteen (3–7, 9–13, *P*). In the eighth segment the appendages have disappeared, and this may be the reason for the absence of the posterior nerves of this segment, which would normally innervate these appendages. The first neuromere has a pair of nerves that innervate the median eyes and a pair of nerves that innervate the lateral eyes. The second neuromere has a pair of nerves that innervate the chelicera and a median unpaired nerve that passes along the œsophagus to the rostrum. This rostral nerve may be a fusion of the anterior nerves of the second neuromere. The cheliceral nerves are serially homologous with the posterior nerves of the segments bearing walking legs. For the same reason I have called the single pair of nerves of the third neuromere posterior nerves. The twentieth neuromere is being drawn up into the nineteenth segment (*n* 20).

Stage 7. — (Figs. 7 and 8.) By a comparison of Fig. 7, which represents the nervous system as more or less transparent, with Fig. 8, which represents a median sagittal section of the nervous system with an outline of the ectoderm, I hope the reader may get a general idea of this stage of the embryo without the necessity of reading much description, and I will call attention only to special points.

The median-eye sacs have come together in the mid-line and open by a common neck (*omo*) to the exterior. Nerve-centers nineteen and twenty have completely fused. In the first neuromere there are two cross commissures, a fact which leads many to suppose that we have here two neuromeres, as does also the fact that it contains two pairs of optic lobes and two pairs of optic nerves. As to the optic lobes: Saint-Remy has shown that they are intimately associated by their nerve tracts. The significance of the optic nerves in relation to the segmentation cannot be determined here, as they cannot be homologized with anterior or posterior nerves of succeeding segments, for, on the one hand, it is doubtful whether their end organs represent appendages and, on the other hand, their roots cannot be traced to the ventral or central columns of Police, as these columns cannot be distinguished in the first neuromere.

Stage 8.—(Fig. 9.) The changes that have taken place since the preceding stage are slight, and may be easily seen by comparing Figs. 7 and 9. It may be noted that the fifteenth nerve center has shifted to the sixteenth segment, the seventeenth nerve center to the eighteenth segment, and that the eighteenth, nineteenth and twentieth nerve-centers lie in the nineteenth segment.

Stage 9.—(Fig. 10.) This represents the embryo at the time of birth. The definitive outer form has been attained but the ganglia are proportionally larger

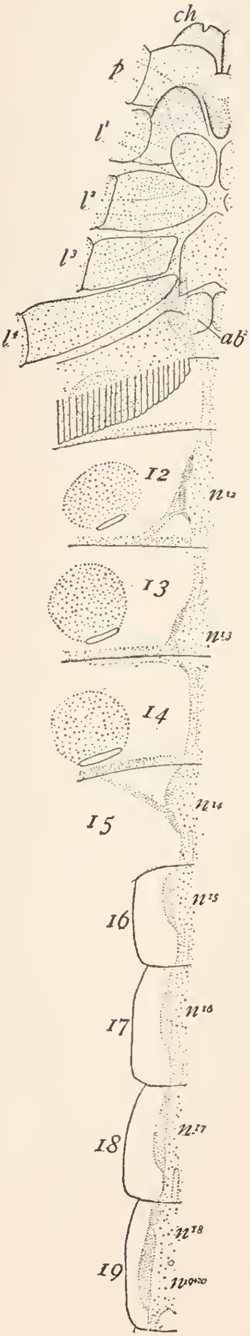

FIG. 10. Ninth stage (at the time of birth), × 34. Drawn with the camera lucida from two cleared preparations, and compared with dissections and series of sections. ab^2, second abdominal appendage forming the genital opercula; ch, chelicera; p, base of pedipalp; l^{1-4}, bases of walking legs; n^{12-20}, twelfth to twentieth neuromeres; $^{12-19}$, twelfth to nineteenth body segments.

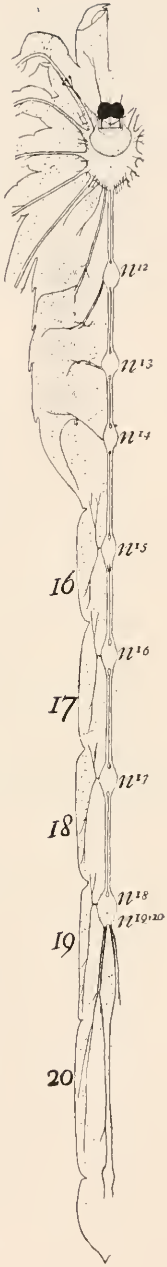

FIG. 11. Tenth stage (adult), × 7. Camera lucida drawing of a dissection from the dorsal side. n^{12-20}, twelfth to twentieth nerve centers; $^{16-20}$, sixteenth to twentieth body segments.

than in older individuals. A further displacement of nerve centers results in that of the fourteenth segment being located in the fifteenth.

Stage 10.—(Figs. 11–13.) This is the adult stage, and since there is little change in the nervous system after the first moult, except increase in size, it will apply equally well to young or old individuals.

I have not been able to add anything to the excellent work of Saint-Remy and of Police (1901, 2) concerning the internal structure of the nerve centers. The nervous system of the scorpion has been dissected and figured by many skilled observers, but in the light of my observations on the embryology perhaps I may call attention to some facts of interest.

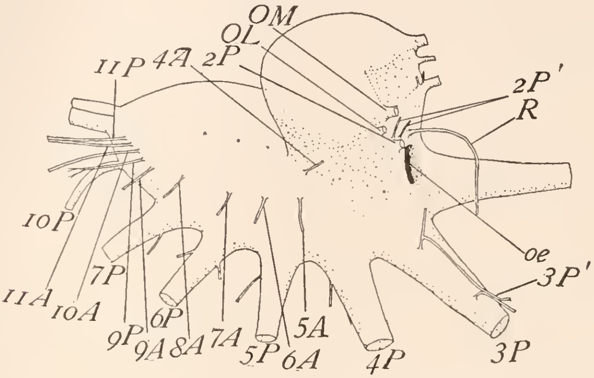

FIG. 12. Tenth stage (adult), × 34. Drawn from a plastic model of the cephalothoracic nerve mass made with the aid of dissections and series of sections in three planes. *OL*, lateral eye nerve; *OM*, median eye nerve; *R*, rostral nerve; $2P$, cheliceral nerve; $2P^1$, branches of cheliceral nerve; $3P$, pedipalp nerve; $3P^1$, branches of pedipalp nerve running parallel a short distance; 4–11*A*, anterior nerves of neuromeres four to eleven; 4–7, 9–11*P*, posterior nerves of corresponding neuromeres.

Each cheliceral nerve (Fig. 12, $2P$) gives off two small branches ($2P^1$) just before piercing the outer neurilemma. Patten figures two small nerves in this position which he calls the "anterior and posterior hæmal nerves" of this neuromere, but from a study of their development I feel certain that they are merely branches of the cheliceral nerve.

Patten describes and figures a large ganglion in connection with a branch of the pedipalp nerve and a branch of the "anterior hæmal" nerve of the same neuromere. What he calls the "anterior hæmal" nerve is another branch of the pedipalp nerve. I have carefully studied the nerves in the region where he described the ganglion (Fig. 12, $3P^1$) but have been unable to find the ganglion.

The nerves to the thoracic appendages and the pectines are larger, and arise from a more ventral part of the cephala-thoracic nerve mass than the other nerves and are classed by Patten as "neural" nerves. Fig. 12 shows how I have classified these nerves from a study of their development better than a description would do. In Fig. 11 I have shown that neuromeres eleven to twenty innervate not only their corresponding segments, but each sends nerves to muscles lying in the succeeding segment. It is possible that the myomeres as well as the nerves are displaced somewhat backward. Patten claims that in the scorpion some nerves wander to segments to which they did not originally belong, and describes certain nerves (Fig. 12, $9A$, $9P$, $10A$, $11A$, $11P$) as "vagus" nerves. Police (1901, 1) denies that this is true, and with the exception stated above, it is not true according to my observations.

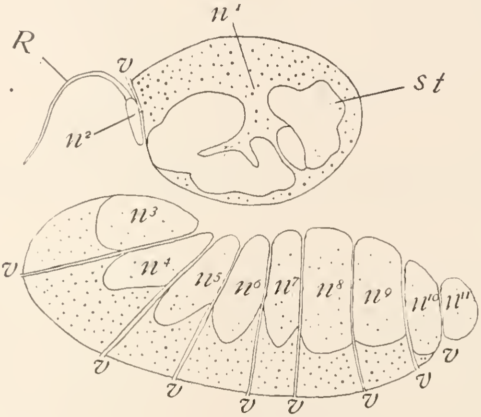

FIG. 13. Tenth stage (adult), × 34. Median sagittal section of cephalothoracic nerve mass. n^{1-11}, first to eleventh neuromeres; *R*, rostral nerve; *st*, stratified organ of Saint-Remy; *vv*, blood vessels between the neuromeres.

SUMMARY OF RESULTS.

In the scorpion there are twenty neuromeres, corresponding to the twenty body segments (not considering the telson as a segment).

The type of neuromere in the early embryo consists of a pair of ganglia fused in the mid-line and two pairs of nerves, anterior (" hæmal " of Patten " external " of Police) and posterior (" neural " of Patten " internal " of Police).

The first neuromere departs strikingly from the typical neuromere as the segment containing it departs from the typical segment. The second neuromere has no anterior nerves unless they have fused to form the rostral nerve. The third neuromere has no anterior nerves. The eighth neuromere has no posterior nerves. The remaining neuromeres are typical.

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'03 Zur Embryologie des Skorpions. Zool. Anz., Bd. 27, pp. 49–58.

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'92 Richerches Comparatives sur l'Organisation du Cervean dans les Principaux Groupes d'Arthropodes. C. R. Soc. Biol., Paris (9), IV., pp. 354–357.

'93 Comparitive Researches upon the Organisation of the Brain in the Principal Groups of Arthropods. Ann. Mag. Nat Hist. (6), Vol. II., pp. 62–65.

'93, 2 Centres Nerveux et les Organes de Sens des Animaux Articules. Ann. Sc. Nat., Paris, 1893.

A LIST OF THE TYPES OF FOSSIL VERTEBRATES IN THE MUSEUM OF THE UNIVERSITY OF TEXAS.

THOS. H. MONTGOMERY, JR.[1]

A considerable number of fossil vertebrates, collected by the geological surveys of the State of Texas under the direction of Mr. E. T. Dumble, state geologist, had been sent to Dr. E. D. Cope for identification. Much of this material is in very fragmentary condition, but Dr. Cope labelled all the recognizable specimens and described from the collection a number of new species. For ten years or more this material has remained in unopened boxes, as it was returned by Dr. Cope. In the course of the past summer I have arranged these collections in the University Museum, and carefully determined all the type material, a comparatively easy task since Dr. Cope had labelled such specimens in almost all cases with the word "type." All the types described by Dr. Cope in the reports of the Geological Survey have been found, with the exception of those of *Pliauchenia spatula* and *Microdus dumblei*.

Since this valuable material has finally been rescued after so many years of obscurity, it seems advisable to publish a list of the type specimens for the use of students of vertebrate paleontology.

In this list I have stated the place of first description of each of the species, with the exception of *Deltodes planidens* and the species of *Mesodon:* these are not described in the Geological Reports, and I can find no mention of them in the "Zoological Record." Most of the species are described in the "Report on the Paleontology of the Vertebrates," Third Annual Report of the Geological Survey of Texas, published in 1892; and in "A Preliminary Report on the Vertebrate Paleontology of the Llano Estacado," Fourth Annual Report of the Geological Survey of Texas, published in 1893. For the sake of brevity these two papers may be referred to respectively as the "Third Report" and the "Fourth Report."

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[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 63.

Pisces.

Deltodes planidens Cope.
Mesodon diastematicus Cope.
M. dumblianus Cope.

Reptilia.

Belodon superciliosus Cope, Fourth Report, p. 12.

Palæoctonus orthodon Cope, Ibid., p. 15.

P. dumblianus Cope, Ibid., p. 16.

Testudo hexagonata Cope, Ibid., p. 77.

T. laticaudata Cope, Ibid., p. 75.

T. turgida Cope, Proc. Amer. Phil. Soc., 1892, p. 127.

T. pertenuis Cope, Ibid., p. 226. The material of this, as of *turgida*, is in very fragmentary condition and not marked "type"; but probably it is a portion of the type material.

Mammalia.

Edentata.

Megalonyx leptostomus Cope, Fourth Report, p. 49.

Tomiopsis ferruminatus Cope, Proc. Amer. Phil. Soc., 1893, p. 317.

Proboscidea.

Dibelodon præcursor Cope, Fourth Report, p. 64.

Tetrabelodon serridens cimarronis Cope, Ibid., p. 18.

Ungulata.

Platygonus bicalcaratus Cope, Fourth Report, p. 68.

Blastomeryx (Merycodus) gemmifer Cope, Annual Report U. S. Geological Survey of Territories, 1874, p. 531. Report U. S. Geological Geography. Surveys west of the 100th meridian, 1877, p. 360.

Holomeniscus macrocephalus Cope, Fourth Report, p. 85.

H. sulcatus Cope, Ibid., p. 84.

Procamelus leptognathus Cope, Ibid., p. 37.

Protohippus fossulatus Cope, Ibid., p. 30.

P. pachyops Cope, Ibid., p. 41.

P. lenticularis Cope, Ibid., p. 41.

Hippidium interpolatum Cope, Ibid., p. 42.
Equus cumminsii Cope, Ibid., p. 67.
E. eurystylus Cope, Ibid., p. 43.
E. semiplicatus Cope, Ibid., p. 80.
E. simplicidens Cope, Proc. Amer. Phil. Soc., 1892, p. 124.
E. minutus Cope, Fourth Report, p. 67.

Carnivora.

Felis hillianus Cope, Fourth Report, p. 55.
Borophagus diversidens Cope, Amer. Nat., 1892, p. 1028. — Fourth Report, p. 54.
Canimartes cumminsii Cope, Fourth Report, p. 52.

University of Texas,
September 24, 1904.

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BIOLOGICAL BULLETIN

DIVERGENCE AND CONVERGENCE IN FISHES.[1]

CARL H. EIGENMANN.

ILLUSTRATIONS BY CLARENCE KENNEDY.

The struggle for existence with the biological environment as the result of the geometric rate of increase tends to divergence in habit and form. It tends to divergence in habit and form by preserving variants whenever such possess a character diverging sufficiently in amount to give the variant a personal advantage over his fellows — always provided the divergent character is transmissible.

Whether we call the diverging individuals variants in the old sense, or mutants in the new, it is to the selection of those among them best adapted to utilize the foods of various sorts, to escape the enemies of various sorts and to leave others similar to them in their place when they die that we owe the specific divergence in structure, shape, color, food-habits and breeding-habits of a given family — say the American characins. The entire process tends to the divergence and multiplicity of species.

The characins are a family of fresh-water fishes that, in America, range from the border of the United States to some distance south of Buenos Ayres. They form about one third of the entire South American fresh-water fauna, and have diverged in adaptation to diverse food, diverse habitat and diverse enemies to fill nearly every niche open to fishes. The ends of the three lines of adaptation to different food give us mud-eating forms, with long intestinal tract and no teeth; flesh-eaters with shear-like teeth, that make bathing dangerous to life and that cut their way out of nets; and conical-toothed forms, with sharp, needle-like teeth and comparatively huge fangs. Greater diversity could scarcely be imagined, and one is lead to suspect that some of the forms are over-adapted. In their divergence in form they have reached almost every conceivable shape as we shall see in a moment.

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[1] Contributions from the Zoölogical Laboratory of the Indiana University, No. 64.

The struggle for existence with any unit of physical environment, whether there be geometric rate of increase or not, tends to convergence in habit and form. There is no more striking instance of this than the acceptance of the annual, or deciduous, habit of most of the plants inhabiting the temperate zones with their seasonal changes. Records of the simultaneous and similar changes in the form in the mass of species of any area during changing physical conditions are not wanting. For instance, Scott says:[1]

"The steps of modernization which may be observed in following out the history of many different groups of mammals are seen to keep curiously parallel, as may be noticed, for example, in the series of skulls figured by Kowalevsky, where we find similar changes occuring in such families as the pigs, deer, antelopes, horses, elephants, etc. Indeed, one may speak with propriety of a Puerco, or Wasatch, or White River type of skull, which will be found exemplified in widely separate orders."

On some riffles of the San Juan river of Cuba I found a small fish that is very strikingly like other fishes inhabiting similar localities in the eastern United States. The former is a goby, a marine form, *Philypnus dormitator*, that has become adjusted to the conditions found about the riffles of streams; the others are darters, *Hadropterus*, belonging to an entirely different family of fresh-water fishes. The similarity of various "darters" which live on the bottom of our streams to various gobies and blennies that occupy a similar position along marine shores has repeatedly been noticed.

In the tropics live many burrowing lizards and snakes. *Rhineura*, one of the lizards, lives and acts like an earthworm and so like an earthworm has it become that only a close inspection reveals its true nature. Even the chickens following the plows in Florida and Cuba are said to be taken in by the similarity of some of the burrowing lizards to earthworms.

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[1] *Journ. Morphol.*, V., 365, 1891.

The characins again furnish striking illustrations. Diverging among themselves, as has been noted above, they have approached, or paralleled many members of the diverse families of North American fresh-water fishes. Our shads and fresh-water herrings have their counterparts in *Elopomorphus*, *Potamorhina* and *Psectrogaster;* our salmon are paralled by *Salminus* and *Catabasis;* our minnows are paralleled by *Tetragonopterus* and its relatives. It will take but a slight flight of the imagination to detect the striking similarity of some of the Hydrocyninæ to our gar-pikes; our mullets are duplicated by *Prochilodus;* our top-minnows are mimicked by *Nannostomus*, and even our festive darters are duplicated by a member of this most remarkable family, *Characidium fasciatum*.

I have elsewhere given an example of convergence under the similar conditions presented by different caves. *Troglichthys rosæ*, a blind fish of southwestern Missouri, resembles so closely *Typhlichthys subterraneus* of Kentucky that at least two naturalists, expert in detecting specific differences in fishes, had failed to distinguish them, although below the adaptive resemblances of the surface there was distinct evidence that the epigean ancestors of the two species were generically distinct.

A cave presents a "unit of environment," a little world, a microcosm, with both positive and negative characters.

1. This unit is well circumscribed and connected with the rest of the universe frequently by but a single narrow vestibule. In this vestibule we have a rapid graduation from external to internal cave conditions — a region which acts as a purgatory for all forms that would enter the elysian fields within. The majority of epigean forms never get beyond the twilight of this region.

2. The feature that distinguishes the interior of the cave is the constant absence of sunlight and all which that implies.

(*a*) The absence of all green plants and consequently the absence of all direct food-producing activity. (*b*) As a result of (*a*) all food must ultimately be imported. (*c*) As a result of (*b*) the abundance of the fauna in different caves is frequently inversely proportionate to the size of the cave, or directly to the ability of the openings to admit food to the various recesses of the different caves.

3. A feature no less striking, though varying greatly in different caves, is the constancy of the temperature — the absence of great diurnal and seasonal changes. This differs very much in different caves, and we have caves in the temperate zone in which there is always ice — in summer as well as in winter — and others in which ice never forms. This difference is due to the shape of the cave and the accessibility of outside air in winter and summer. Usually seasonal changes are very slight.

4. This unit of environment is further characterized by the relatively stationary atmosphere, the absence of rain, snow, or severe currents of air. In the deeper recesses of a cave it is rarely that an air-current is perceptible with an anemometer unless there is moving water near. In the blind-fish caves of Mitchell, Indiana, a current of air enters the cave above with the water and leaves it three quarters of a mile below with the water. In Little Bat avenue, one of the upper tiers of river channels of Mammoth Cave, there is no perceptible air-current until the top of Mammoth dome is approached, where a perceptible current enters the dome, descending to the bottom of the dome and then ascending again to leave the dome about half way up by Sparks avenue. This local air-current is caused by the cascade descending from the uppermost to the lowermost part of the dome.

Air-currents are most readily perceived at a cave's entrance and vary with the size of the opening, the size of the cave and the rapidity of barometic changes on the outside.

In a small cave air-currents are not strong about the entrance. In a large cave, such as Mammoth, where the opening is small the current may become a fierce gale if barometric changes are rapid or if the water in the cave rises rapidly. If the barometric pressure increases there will be an inflow of air and this will be proportionate to the rise of the barometer and to the size of the cave. If the barometer is falling there will be an outpour of air and the pressure on the inside or outside may be so nicely balanced that no current will be perceptible, or there may be a continued shifting of currents in or out every few moments with every slight shifting of outer atmospheric pressure not perceptible with the ordinary mercurial barometer.

5. What has gone before concerning the changes in light, heat and plant-growth in the cave in general applies to the water in a cave except that the total content of the rivers of a cave will change with comparative rapidity. With every freshet in epigean rivers there is a corresponding freshet in the subterranean streams. There must be some change in the temperature of the water in caves though the change is not at all commensurate with the changes in temperature in epigean rivers. No detailed records of water temperatures are at hand.

In an environment, such as that above described, all those differences between related species which would strike the eye, such as protective coloration, recognition marks, decorations of any sort, etc., are absent and related species tend to look alike. *Amblyopsis* and *Typhlichthys* look alike when of the same size, and it was not until after a detailed examination of many specimens that I could invariably distinguish *Lucifuga* and *Stygicola*, the Cuban blind fishes, from each other. There are two unrecorded species of *Typhlichthys*, differing from the only known species, *subterraneus*, in only a few inconspicuous respects.

Typhlichthys subterraneus has been known chiefly from Mammoth cave, the type having come from a well at Bowling Green, south of Mammoth Cave. I have repeatedly taken it at Glasgow, Kentucky. It has erroneously been recorded from Missouri and Indiana, but is confined to the region south of the Ohio and east of the Mississippi.

A number of years ago a single specimen of *Typhlichthys* was sent to Indiana University from Corydon, Ind., and it was referred to *subterraneus*. Every endeavor to secure additional specimens has so far failed. The Corydon specimen may here be described as

Typhlichthys wyandotte Eigenmann, sp. nov.*

The single specimen taken from north of the Ohio River, from a well near Corydon, Indiana, differs slightly from those south of the Ohio, being somewhat more slender. The Corydon specimen is 42 mm. in length from the tip of the snout to the base of the caudal, and other measurements are as follows : Head in length of body to base of caudal, 3⅔ ; width of head in length of body 6½, 1⅔ in its own length ; distance from posterior

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*This specimen has been recorded as *Typhlichthys subterraneus* Eigenmann, Proceedings Indiana Academy of Science, 1898 for 1897, 230 (Corydon, Indiana).

margin of the skull to the front of the first dorsal ray, 16 mm.; front of dorsal to middle ray of the caudal, 17 mm. First anal ray nearer base of middle caudal ray than the anus. Specimens from about Mammoth Cave 42 mm. long, measure as follows: Head 3 to 3¼ in length of body; width of head in length of body, 5, $1\frac{1}{2}$ to $1\frac{3}{5}$ in its own length; distance from the base of the skull to the first dorsal, 15 mm.; front of dorsal to middle ray of caudal, 17½ mm. First anal ray about equidistant from the base of the middle caudal ray and the anus.

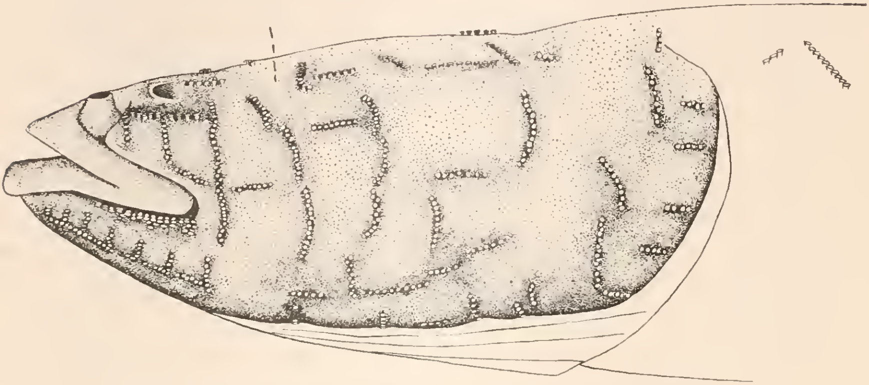

FIG. 1. Side view of the head of a *Typhlichthys subterraneus.*

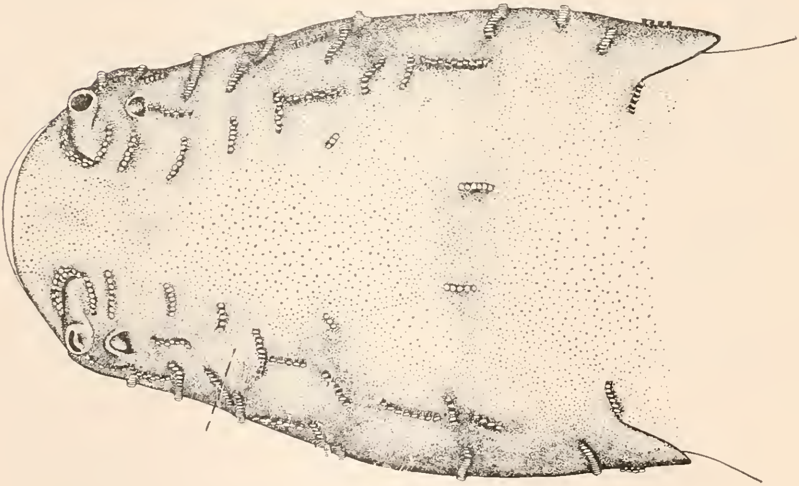

FIG. 2. Dorsal view of the same.

The second new species may be described as

Typhlichthys osborni * Eigenmann, sp. nov.

November 29 to December 2 a large number of *Typhlichthys* were obtained. Five from Mitchell's cave at Glasgow representing *subterraneus* and forty-two from Horse Cave. Both these localities are on the L. & N. R. R., not far from the Mammoth Cave. The specimens from the two locali-

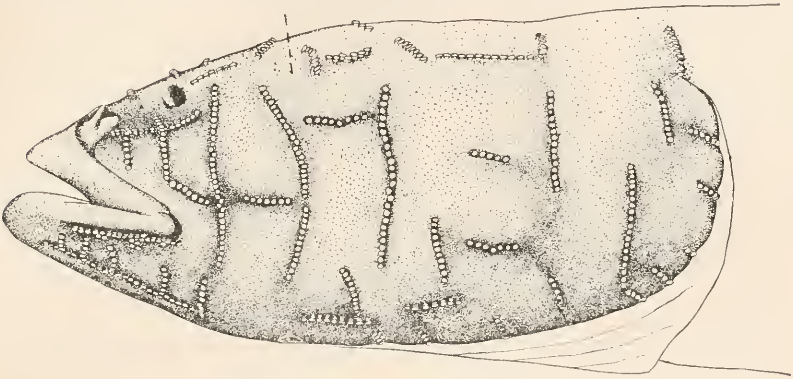

FIG. 3. Side view of the head of a *Typhlichthys osborni.*

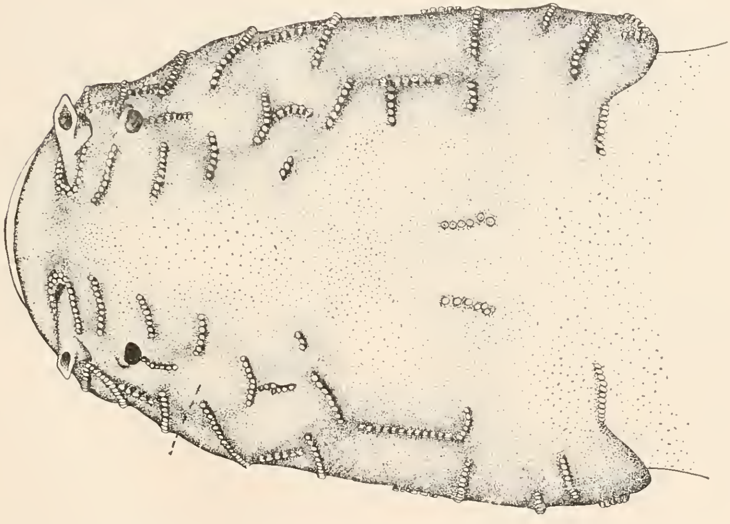

FIG. 4. Dorsal view of the same.

All the figures show the tactile organs of the head and their mode of arrangement. The general outlines and the arrangement of the tactile organs are from camera sketches, the number of tactile organs are from actual counts. The differences in the eye region can be but faintly indicated in the drawings.

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* To Henry Fairfield Osborn of the American Museum of Natural History in recognition of his interest in the zoölogy of the interior of the United States.

ties differ strikingly while alive. In the Horse Cave specimens the head is pointed, the cheeks puffed, the eye spaces show conspicuously as white spots and bulge out like a rounded dome, the fatty masses above and below on caudal peduncle are conspicuous and white. They measure 20–50 mm.

The specimens from Glasgow have the eye-spaces inconspicuous, not protruding, and the caudal fatty masses are inconspicuous. The largest specimen from Horse Cave agrees with these in most respects.

These specimens were put into two aquaria with siphon overflow of 5 and 10 mm., into a common central aquarium. The specimens readily moved through the siphons from one to the other. One specimen had traversed both siphons in between two and three hours.

The Horse Cave specimens stay under leaves, etc., in their aquarium. Those from Glasgow swim about more.

The characters of the three known species of *Typhlichthys* may be summarized as follows:

(*a*) Width of head more than 6 in the length to base of caudal; length of head $3\frac{2}{3}$; first anal ray nearer base of middle caudal ray than to the anus...*wyandotte.*

(*aa*) Width of head 5 in the length to base of caudal; length of head $3–3\frac{2}{5}$; orbital fat-mass elongate, inconspicuous in life, not projecting; cheeks little swollen; eye on an average .16 mm. in diameter, the smallest measures .14 mm. *subterraneus.*

(*aaa*) Width of head $4\frac{1}{2}$ in the length to base of caudal; length of head $3\frac{1}{6}$; orbital fat-mass round and very conspicuous in life, projecting dome-shaped beyond contour of surrounding parts; cheeks much swollen; eye less than .10 mm. in diameter..........*osborni.*

THE RANK OF NECTURUS AMONG TAILED BATRACHIA.[1]

B. F. KINGSBURY.

During the years 1901–1903, I had the opportunity of examining in the Anatomisches Institut of Freiburg i/B., a large number of series through the head of *Necturus*, *Spelerpes* (larval and adult) and *Desmognathus* (larval and adult). I was immediately struck by the very close resemblance between the larval *Spelerpes* and *Necturus*, and drew up the following table, which it seems to me possesses sufficient value to warrant its publication with brief comments. I have rendered it more complete by including in the comparison other forms which must necessarily be considered in this connection, *i. e.*, Axolotl, Siren, Typhlomolge. *Proteus* of course agrees with *Necturus* in all essential points. In addition to information gained from specimens, I have made use of the papers of Wiedersheim,[2] Parker,[3] Cope[4] and Stejneger.[5] Especially valuable will be further information as to the cranial characters of *Typhlomolge*. Further work on the cranial anatomy of Siren and the Axolotl would also be important for such a comparison as the following.

Many, perhaps most of the facts presented below are already known but I think their significance is not sufficiently recognized.

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[1] I shall use the term "Urodele" and "Urodela," as synonymous with "tailed Amphibian" in this paper, although I realize that such a usage is not quite correct.

[2] 77. Wiedersheim, R., "Das Kopfskelet der Urodelen.," *Morph. Jahrb.*, Bd. III., pp. 352–548.

[3] '77. Parker, W. K , "On the Structure and Development of the Skull in the Urodelous Amphibia," Pt. I., *Philos. Trans. Roy. Soc.*, Vol. 167, Pt. 2.

'82*a*. *Ibid.*, "On the Structure and Development of the Skull in the Urodeles," *Trans. Zool. Soc. London*, Vol. XI., pp. 171–214.

'82*b*. *Ibid.*, "On the Morphology of the Skull in the Amphibia Urodela," *Trans. Linn. Soc.*, Ser. 2, Vol. II.

[4] Cope, E. D., "The Batrachia of North America," *Bull. U. S. Nat'l. Museum* No. 34, 1889.

[5] Stejneger, L., "Description of a New Genus and Species of Blind Tailed Batrachian from the Subterranean Waters of Texas," *Proc. U. S. Nat'l. Museum*, Vol. XXVIII.

A COMPARISON OF CERTAIN CRANIAL CHARACTERS IN NECTURUS, LARVAL AND ADULT SPELERPES, AMBLYSTOMA AND THE AXOLOTL, SIREN, AND TYPHLOMOLGE.[1]

Character.	*Necturus.*	*Larval Spelerpes.*	*Adult Spelerpes.*	*Amblystoma.*	*Axolotl.*	*Siren.*	*Typhlomolge.*
Frontals, Parietals, Squamosals, Premaxillaries, Parasphenoids.	Present.	Present.	Present.	Present.	Present.	Present.	Absent.
Vomer.	Present.	Present.	Fused with Pal.-pter.	Fused with Pal.-pter.	Present.	Present.	
Vomero-palatine.	Absent.	Absent.	Present.	Present.	Absent.	Absent,[2] but bones close.	
Palatine.	See Pal.-pter.	See Pal.-pter.	Fused with Vomer.	Fused with Vomer.	Present. Pal. pter.	Present.	
Pterygo-palatine.	Present.	Present.	Absent.	Absent.	Present?	Absent.	
Pterygoid.	See Pal.-pter.	See Pal.-pter.	Absent.	Present.	Present?[3]	Absent?[4]	
Quadrate and bone "X."	Partly ossified.	Partly ossified.	Entirely ossified.	Ossified.			
Nasals.	Absent.	Absent.	Present.	Present.	Present, thin.	Present.	
Prefrontals.	Absent.	Absent.	Present.	Present.	Present?	Absent.	
Maxillary.	Absent.	Absent.	Present.	Present.	Rudimentary.	Absent.	
Pterygoid process of quadrate.	Absent.	Absent.	Present.	Present.	Present.	Short.	
Nasal capsule.	Imperfect, not connected with cranium.	Absent.	Present, complete, connected with cranium.		Extensive.	Well developed.	
Dentare,	Present.	Present.	Present.	Present.	Present.	Present.	
Angulare,	Present.	Present.	Present.	Present.	Present.	Present.	
Articulare.	Absent (as such).	Absent (as such).	Present and fused with angulare?				
Operculare (Spleniale).	Present.	Present.	Absent.	Absent?	Present.	Present?	
Intermaxillary gland.	Absent.	Absent.	Present.	Present.		Present.	
Muscles and ligaments.	In *Necturus* and *Spelerpes*, morphologically the same.		Altered in metamorphosis.				
Nerves of head.	In *Necturus* and larval *Spelerpes*, nearly morphologically the same.		Altered slightly in metamorphosis.				

[1] In the above table, the interrogation point indicates that the statement made in the literature needs careful verification at first hand. A "blank" indicates that nothing is known to the writer concerning the point in question.

[2] Both vomer and palatine are present and close, but they are not coösified. The Pterygoid portion of the original Palato-pterygoid, seems to be wanting.

[3] Since we cannot be sure whether the Mexican Axolotl was the form worked on, or the larva of *Ambystoma tigrinum*, descriptions by Parker and Wiedersheim are unsatisfactory.

[4] The palatine portion is present, but the pterygoid portion has apparently been absorbed.

The first five bones mentioned in the above table need no comment since they are universally present. Of the other "roof bones" of the Urodele skull, the nasals and prefrontals which are present in the gill-less adults, are completely lacking in *Necturus* and in the larval *Spelerpes*, and seem to be absent in the larvæ of other forms (as *e. g.*, *Diemyctylus*, Parker, 82*a*, p. 178), or but slightly developed (*Salamandra*, Parker, 82*b*). Quite characteristic is the occurrence in the larvæ of Urodela of a Pterygo-palatine (or palato-pterygoid) extending from the ventral side of the quadrate cartilage to the roof of the mouth where as the palatine portion, it is typically tooth-bearing. In the adult gill-less forms, the palatine portion becomes separated from the pterygoid portion and fused with the vomer forming a new osseous skeletal unit, the Vomero-palatine. The Pterygoid portion may be absorbed completely, or remain as a distinct bone, the Pterygoid.

The Maxillare is lacking in the larval forms or if present, as it seems to be in advanced *Amblystoma* larvæ and the Axolotl, it is but weakly developed. In all gill-less adult Urodela it is present.

The quadrate in *Necturus* is peculiar. The lower end, which articulates with the mandible is ossified, while the body of the Quadrate and the otic, trabecular and basilar processes remain cartilaginous. Ossification first appears in a bone, which I call here bone "X"[1] upon the upper (dorsal) surface of the quadrate cartilage, slightly before hatching. It appears to be at first entirely distinct from the cartilage. The lower end soon becomes closely applied to the quadrate cartilage and finally fused with it. From this (as a center?) a zone of ossification spreads inside and outside around the lower end of the cartilage, forming a ring of bone. It was interesting to find in the larval *Spelerpes* a precisely similar method of ossification, identical, it seemed, in all respects with that in *Necturus*, save that the primary ossification was more closely associated with the cartilage. In the larva, the ossification does not proceed beyond the stage found in *Necturus*. The bone "x" coössifies at its distal end with the lower end of the quadrate, but proximally is free from it. In the adult *Spelerpes*, the entire quadrate cartilage, with the exception of the articular surfaces, has become ossified, with the formation of a marrow cavity, and the primary ossification, bone "x," is no longer distinguishable as a distinct structure. In how far this same mode of ossification prevails among Urodela, I cannot say, but I believe from examination of *Amblystoma*, *Amphiuma* and *Desmognathus*, quite generally.

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[1] Cf. Kingsbury, B. F. "Columella Auris and Nervus Facialis in the Urodela," *Journ. Comp. Neurol.*, Vol. XIII., No. 4, 1903.

A cartilaginous process of the quadrate is quite generally found in Urodela extending forward toward the antorbital process of the chondrocranium, forming a "pterygoid arch"(?). Its absence in *Necturus* is noteworthy. It is also absent in the larva of *Spelerpes*, and I believe, most larval urodeles, developing at transformation. Examination of the sections strongly suggested that in both larval *Spelerpes* and *Necturus*, it was present in a "pro-cartilaginous" form.

The Nasal capsule in *Necturus* (and *Proteus*) is quite unique, fenestrated, thin, imperfect, without cartilaginous or bony connection with the chondrocranium. In the larval *Spelerpes* it is absent, developing at transformation.

In the lower jaw, there are comparisons of interest. Briefly stated, the mandibles of *Necturus* and of the larval *Spelerpes* are directly comparable. Each consists of three bones, dentare, operculare (spleniale ?), and angulare together with Meckel's cartilage. The angulare seems slightly fused with the articular head of Meckel's cartilage in both *Necturus* and the larval *Spelerpes*. It extends back, forming the "angle" of the jaw, and to its caudal end attaches the mandibulo-hyoid ligament. In the adult *Spelerpes* this bone and the head of Meckel's cartilage are coösified, forming one bone, which perhaps should be termed articulo-angulare, though I believe that the ossification proceeds from the angulare and is at first distinct from the cartilage, in a manner entirely comparable with the ossification of the articular end of the quadrate cartilage. The operculare, a bone present in larval and absent (always ?) in adult gill-less life, is, interestingly enough, present in *Necturus*. As to its fate in the adult (gill-less), there is at present, I believe, no satisfactory evidence. I do not believe, however, that it becomes fused with the angulare.

The statements of the relation of muscles, ligaments and nerves can only be given in general, as my notes fail me for details. The nerves especially indicated are the V. and VII., and the muscles, the muscles of the jaw, hyoid, and branchial apparatus. I regret also that the table is incomplete in the important point—the relations of the branchial arches—so that I cannot state definitely whether there is complete correspondence, *i. e.*, whether the relations of the second basibranchial and the three epibranchals, are the same or not in the two forms.

As cranial characters peculiarly associated with the larval state, are to be mentioned:

1. Presence of a pterygo-palatine and absence of a vomero-palatine.
2. The absence of prefrontals (and nasals).
3. The absence of a processus pterygoideus quadrati (cart.).
4. The absence of a maxillare.
5. Presence of an operculare (spleniale).

All of these characters *Necturus* possesses, and in addition, closely resembles the larval *Spelerpes* in (*a*) the state of ossification of the quadrate, (*b*) the ossifications in the mandible, and (*c*) less clearly, in the rudimentary development of the nasal capsule.

Four possible interpretations of the structural characters mentioned above, and the systematic position of *Necturus*, may be made. (*a*) *Necturus* is a permanent larva, (*b*) it is a degenerate form. (*c*) It is a generalized or low form and (*d*) the possibility should also be considered that *Necturus* owes the structural characters of the skull in which it resembles larval urodeles, to the fact that both are living under very similar conditions of environment — that these characters are in some way connected with the branchial respiration and aquatic life of the form, and represent an adaptation, and adjustment to the environment.

In favor of *Necturus* as a permanent larva stand the larval resemblances enumerated above. Circumstantial support for this view is also given by the generally accepted fact that the gilled Axolotl (*Siredon pisciformis*) is a permanent larval form of a species belonging to the genus *Amblystoma*.

The statement in Cope's Batrachia, still holds good, I think. No one has accomplished the transformation of the Mexican Axolotl. On the other hand, the larvæ of *Amblystoma tigrinum* often grow[1] to enormous size, and undoubtedly are sexually mature while still possessing the larval form (Cope, Osborne[2]), so that the evidence is good that the Axolotl is a permanent larva.

A tendency to superlarvation seems to exist in other families of tailed Amphibia. *Spelerpes* larvæ attain a length of 60 mm. and I believe an age of two or three years before transforming. Small adults are often of smaller size. *Gyrinophilus porphyriticus*, of the same family, attains a length of twelve centimeters before undergoing transformation. The Museum of Cornell University contains such a specimen, together with adults fourteen centimeters long.

Were the interpretation of *Necturus* as a permanent larva to be seriously accepted, there is no existing form to which it could be related. As far as cranial characters go, the Plethodontidæ, (*e. g.*, *Gyrinophilus*) are temptingly adapted for serving as the transformed kinsmen. However, one very important feature, absence of lungs, of course excludes them from consideration.

The evidence that *Necturus* is a degenerate form, seems to me very slight. There is no reason for regarding the absence of prefrontal, nasal, and maxillary as indications of degeneration. The imperfect condition of ossification about the mandibular articulation also does not seem to me to indicate degeneration. The chondrocranium is much "reduced" in extent; the trabeculæ delicate, the basilar plate imperfect, the nasal capsule incomplete — all of which suggests a reduction from the more usual urodele type. But the place of the chondrocranium has been taken by the bony elements of the skull. The parasphenoid surrounds the cephalic end of the notochord, the frontals and parietals come down to the parasphenoids to complete the side walls of the cranial cavity, so that there is formed a very strong firm skull. From one point of view, perhaps, were *Necturus* regarded as a permanent larva, the fact of superlarvation might itself be regarded as an indication of degeneration. The failure to undergo transformation could be interpreted as, in a sense, arrested development — a form of degeneration.

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[1] '03, Powers, J. H., "The Cause of Acceleration and Retardation in the Metamorphosis of *Amblystoma tigrinum;* A Preliminary Report," *Am. Nat.*, Vol. XXXVII., pp. 385–410. 1903.

[2] '00, Osborn, H. L., "A Remarkable Axolotl from North Dakota," *Am. Nat.*, Vol. XXXIV., pp. 551–562. 1900.

'01, *Ibid.*, "On Some Points in the Anatomy of a Collection of Axolotls from Colorado and a Specimen from Northern Dakota," *Am. Nat.*, Vol. XXXV., pp. 887–897. 1901.

The evidence that *Necturus* is a low form, seems to me quite as tentative in character as that in favor of *Necturus* as a permanent larva. Cope has placed *Necturus* and *Proteus* in an order, the Proteida, as the lowest of the tailed Amphibia, giving as the critical character the presence of an intercalary bone, relating it to the extinct Stegocephali. Examination of series through the skull, however,[1] shows no such bone present. Apparently what was regarded by Cope as an Os intercalare (Epioticum) is the backward extension of the caudal ossification of the ear capsule (opisthotic ?). From examination of figures (no specimens of Stegocephali have been seen by me), the intercalary seems to be a dermal bone and not a part of the ossification of the ear capsule. The ossification in this region is peculiar, consisting, in *Necturus* of a cephalic ossification (proötic ?), and a caudal (opisthotic ?) which perhaps spreads to it from the exoccipital arch. The homology of the ossifications in the ear capsule in Amphibia as elsewhere, I judge to be in a very unsatisfactory state at present, and even though the Os intercalare belonged to the bones of the ear capsule, it would not be of value in determining the rank of *Necturus* until more is known of the mode of ossification in other tailed Amphibia.

So far as the skull is concerned, the real characters which must be considered in placing *Necturus* low among tailed Amphibia, are, I believe, the same as those which, differently interpreted, afford evidence of *Necturus* as a permanent larva, *i. e.*, the absence of prefrontal, maxillary, etc. If we interpret the larval stage as representing in ontogeny a stage through which the species has passed in its development, then larval characters can be regarded as primitive, and *Necturus* as a primitive form. Aside from the general question of "Ontogeny an Epitome of Phylogeny," such an interpretation of the larval stage is not entirely satisfactory ; there are some features that suggest that it may be an adaptation. Furthermore, the "ancestor of the Amphibia" would not be expected to be a form with a reduction in the number of cranial bones, but rather the reverse.

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[1] There were included specimens 16 mm. long up to an individual 20 cm. in length.

It would seem to me worth while to compare other parts of the anatomy of *Necturus* with the larval and adult forms of other urodeles, to see whether there too similar larval characters are to be encountered.

What applies to *Necturus* applies to other gill-bearing forms. If *Siredon pisciformis* never transforms and is yet regarded as a permanent larva, there is no good reason known to me why *Necturus* should not be also considered in the same light. Of the other forms *Proteus*, of course, agrees with *Necturus* in all essential characters. *Typhlomolge* will probably resemble these two when its skeletal characters are made known. Mr. Stejneger in his description of the form says that the maxillary bone is absent and that an intercalary seems to be present. Mr. Lucas is working upon the structural features of the skeleton. His results have not been published as yet, I believe, and will be awaited with interest. *Siren* has been regarded by Cope as a larval form by retrograde metamorphosis. It resembles an "ideal larva" in three of the characters given above — absence of a vomero-palatine, absence of prefontals, absence of a maxillare.

I believe that the characters in the cranium of *Necturus* that are discussed here are those that are also characteristic of the larvæ of many tailed Amphibia, especially so of certain forms (family Plethodontidæ), and that the acceptance of *Necturus* as a permanent larva best explains at present these features.

It is recognized that there are objections to be met. *Necturus* probably can never be made to transform so that proof cannot be furnished. There is no gill-less adult salamander whose larvæ *Necturus* so closely resembles as does *Siredon pisciformis* the *Amblystoma* larva. *Necturus*, *Proteus* and possibly *Typhlomolge* are very closely alike in structural features, are sexually mature, and occur in different parts of the world. One would not expect them to be permanent larvæ.

An attempt to reach a closer decision leads to a study of metamorphosis in the Amphibia.

Cornell University, Ithaca, N. Y.,
October 12, 1904.

A NEW MYZOSTOMA, PARASITIC IN A STARFISH.

WILLIAM MORTON WHEELER.

Mr. Walter K. Fisher, editor of *The Condor*, recently sent me a *Myzostoma* which he took from the cœlomic cavity of a new species of starfish, *Tosia* (*Pentagonaster*) *leptoceramus* Fisher, from Station 4427 off the coast of southern California. The starfish is an extremely flat species and the myzostome was situated in one of the interradial areas, near the gonad, where the body of the host was only 1.5 mm. thick. The parasite appeared to be loosely fastened to the cœlomic epithelium by one end and was greatly flattened, in adaptation to the narrow body-cavity of the starfish.

Comparison of the specimen with the descriptions of the only other known starfish *Myzostoma* (*M. asteriæ*) shows so many differences that I do not hesitate to regard the Californian species as distinct. *M. asteriæ*, originally described by von Marenzeller,[1] occurs in an hypertrophied portion of the digestive tract in the base of one of the arms of certain small starfishes (*Asterias richardi* E. Perr. and *Stolasterias neglecta* E. Perr.) that have been dredged from a depth of 160–710 meters in the eastern Mediterranean.[2] A very careful anatomical and histological study of this species has been recently published by von Stummer-Traunfels[3] so that it is an easy matter to compare the Californian with the Mediterranean species.

MYZOSTOMA FISHERI sp. nov.

The specimen is white. It resembles *M. asteriæ* in size and form, being elliptical and broader than long. It measures 6 mm. in length and 8.5

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[1] "*Myzostoma asteriæ* n. sp., ein Endoparasit von Asterias-Arten," *Anzeig. k. Akad. Wiss. Wien.*, No. 18, Juli, 1895.

[2] von Marenzeller, E. "Berichte der Kommission für Tiefseeforschungen. XVI., Zoolog. Ergebnisse. V. Echinodermen," *Denkschr. d. math. naturw. Kl. d. k. Akad. Wiss. Wien.*, 62 Bd., 1895, pp. 123-148, 1 Taf.

[3] "Beiträge zur Anatomie und Histologie der Myzostomen: I. Myzostoma asteriæ Marenz." *Arb. a. d. Zool. Inst. zu Graz*, 6. Bd., No. 8, 1903, pp. 263–363, Taf. 34–38.

mm. in breadth. The dorso-ventral diameter is only 0.5 mm., and is rather uniform both in the center and at the edge of the body. There are no traces of cirri along the somewhat undulating margin. The five pairs of parapodia (*pm.*) are very small and vestigial. They are neither retracted nor do they project above the ventral surface of the body, although they have distinct setæ. The lateral organs ("segmental sacs") are very small, but clearly developed. A ninth unpaired organ could not be detected. The oral orifice is on the ventral surface and rather near the anterior margin, the cloacal orifice on the dorsal surface a short distance behind the center of the body. The pharynx is well developed, the stomach, or succeeding portion of the alimentary tract, is very long and gives off on either side near its posterior end two main gut diverticula. These subdivide near their bases and again near their tips (*g.d.*). All these diverticula are robust, well-developed and extend to very near the margin of the body. The ovary (*ov.*) is located in the body cavity, to the floor of which it is at-

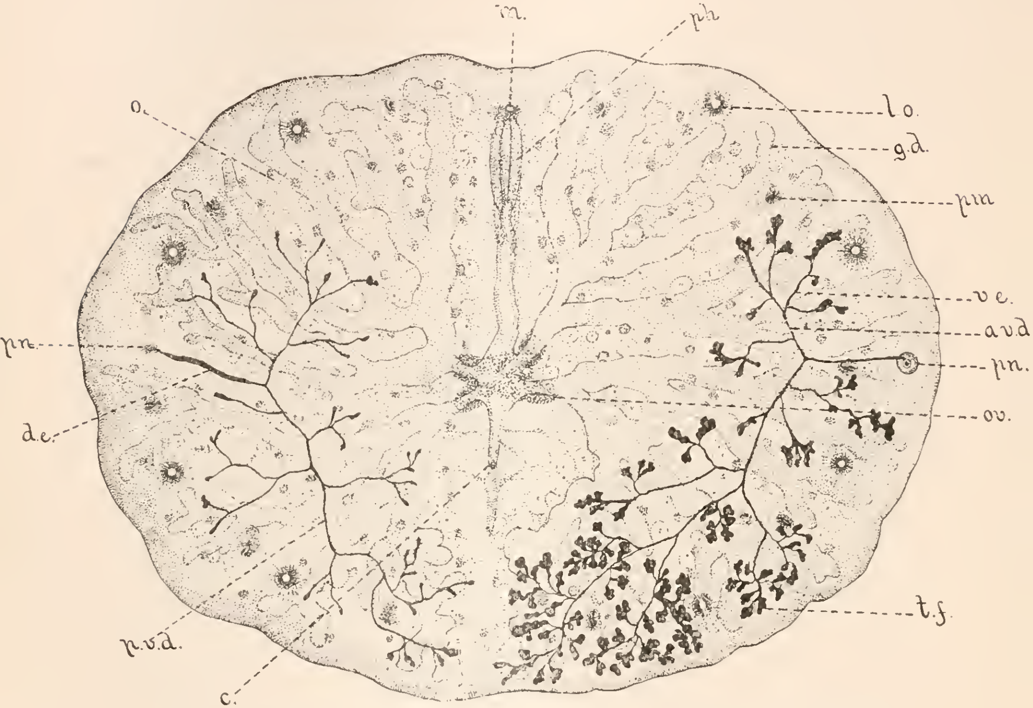

FIG. *A.* *Myzostoma fisheri* sp. nov. Ventral aspect. *m.*, mouth; *ph.*, pharynx; *g.d.*, gut diverticulum; *c.*, cloacal orifice (on dorsal surface); *l.o.*, lateral organ; *pm.*, parapodium; *pn.*, penis; *d.e.*, ductus ejaculatorius; *a.v.d.*, anterior vas deferens; *p v.d.*, posterior vas deferens; *v.e.*, vas efferens; *t.f.*, testicular follicles; *o.v.*, ovaries; *o.*, mature ovum.

tached, on the dorsal surface of the stomach. It is in a very active stage of proliferating the cell-triads (young oöcytes with their attendant nurse-cells). It is a single, unpaired organ of somewhat irregularly cruciform outline when seen in the entire stained and cleared specimen. The body-cavity which is developed only on the dorsal side of the gut-diverticula and accompanies them in their ramifications, contains numerous ova (*o.*) in all stages of growth, attached to the much flattened peritoneal epithelium. The male reproductive organs present a very interesting structure and are in different stages of development on the two sides of the body. On the left side (right side of figure) these organs are highly developed and in full activity, whereas on the left they are much atrophied. Each consists of a penis (*pn.*) which has the form of a small conical papilla on the left, but has all but disappeared on the right side, a *ductus ejaculatorius* (*d.e.*) which extends medially and divides into a shorter anterior and longer posterior vas deferens (*a.v.d.* and *p.v.d*). Each of the *vasa deferentia* again divides into slender *vasa efferentia* (*v.e.*) which terminate in the testicular follicles (*t. f.*), well-developed and still containing mature spermatozoa on the left side of the body, but exhausted and vestigial on the right. The ducts and nearly all the follicles are situated on the ventral side of the gut-diverticula. The specimen may be interpreted, in conformity with my views on the sexual phases of *Myzostoma*, as about to pass from the functional hermaphroditic to the purely female adult stage.[1]

The nephridia, "uterus" and cloaca resemble the corresponding structures in *M. asteriæ* as figured by von Stummer-Traunfels (Taf. 34, figs. 4–9). The short, curved nephridia open on either side by means of distinct nephrostomes into the "uterus" (median body cavity) and by means of their nephridiopores into the cloaca, there being no unpaired end-piece as in some other species of *Myzostoma*. The cloaca is, however, much shorter than in *M. asteriæ*. The "uterus" opens into it very near its orifice on the dorsal surface of the body. The lateral organs also resemble those of *M. asteriæ* in histological structure. The musculature of the body and parapodia is very feebly developed, but the mesenchyma of the former is abundant. The ventral nerve-ganglion lies in the middle of the body beneath the stomach as in other myzostomes. It, too, is very small compared with the nerve-ganglion of *M. asteriæ*.

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[1] These views on the protandric and hysterogynic nature of *Myzostoma*, advanced in my papers entitled: "Protandric Hermaphroditism in Myzostoma," *Zool. Anzeig.*, No. 447, 17, Jahr. 9, pp. 178–182; and "The Sexual Phases of Myzostoma," *Mitth. Zool. Staz. Neapel.*, 17 Bd., 2 Heft, 1896, pp. 227–302, Taf. 10–12; gave rise some years ago to an unpleasant controversy with John Beard, who persisted in maintaining the existence of complemental males among the Myzostomidæ. It is a great pleasure, after the lapse of a decade, to find that my views have been completely confirmed by so careful an investigator as von Stummer-Traunfels. In a forthcoming paper on several new species of *Myzostoma* I shall produce still further proof of the correctness of my contention.

It is evident that *M. asteriæ* and *M. fisheri* are in many respects much more closely related to each other than to any other described species of the genus. Both species agree in the flattened, transversely elliptical form, complete absence of cirri, diffuse ramification of the male reproductive organs, position of the ovary and cloacal orifice, structure of nephridia, etc. On the other hand, they differ in the following important characters: (1) *M. fisheri* is white, whereas *Asteriæ* is buff-colored; (2) the parapodia of the former species do not project above the surface of the body and are very much smaller than they are in the latter; (3) the lateral organs, too, are very much smaller in *fisheri* than in *asteriæ* and there is no ninth unpaired organ in the former species; (4) the pharynx of *fisheri* is much longer, the mouth nearer the anterior edge of the body; the gut diverticula are more robust and less branched at their tips; (5) in *asteriæ* the testes and their ducts are very differently arranged. They are not largely confined to the posterior half of the body, the ducts are much more robust than the terminal follicles (to judge from v. Stummer-Traunfels' Fig. 10, Pl. 34) and there are not two main *vasa deferentia;* (6) the ovary of *asteriæ* is paired, that of *fisheri* unpaired; (7) the Mediterranean species lives in the alimentary tract, the Californian species in the body cavity of the starfish. In this respect *fisheri* is quite unique among the known myzostomes. The other species of the group are either free ectoparasites on crinoids, like *M. cirriferum*, or inhabit the pharynx of their host (*M. pulvinar*) or its gut diverticula (*M. asteriæ*) or live in cysts or galls which they produce in its body wall or skeleton (*M. cysticolum* et al.). In his letters Mr. Fisher is quite explicit in regard to the position of *M. fisheri* in the cœlomic cavity of its host, so that there can be little doubt about the fact.

American Museum of Natural History,
Oct. 27, 1904.

A SIGHT REFLEX SHOWN BY STICKLEBACKS.

WALTER E. GARREY.

The casual observer of the aquaria of the United States Fish Commission at Woods Hole, Mass., always evinces considerable interest in the behavior of the schools of sticklebacks (*Gasterosteus bispinosus*) which are exhibited annually, the attention being attracted by the very characteristic way in which these little fish swim about. During the summer of 1900, while at the Marine Biological Laboratory, the author made some observations bearing on these movements and extended them during the summer of 1904.[1]

In walking past the aquarium it was noticed that all the fish were oriented with the long axes parallel and that the whole school swam in a course parallel to, but in a direction opposite to that of the moving observer. When the observer stops the fish may also stop, but usually continue swimming in the same course, although at a slower rate, till they reach the end of the aquarium, where they usually remain as long as the observer is quiet. If the observer retraces his steps they again orient themselves and swim back to the other end of the aquarium. At first sight this looks like simple fright with movements limited by the walls of the aquarium, but this is not the case as the following experiments will show:

If the observer remains stationary opposite the center of the aquarium and moves some object, preferably a white one held in the hand, horizontally, the little fish at once respond, show a most beautiful orientation and movement, slow and uniform, opposite to that of the moving object. This movement can be arrested at any instant, or the path of motion completely reversed by moving the light object in the opposite direction. This reversal may be produced when the fish are in the center of the aquarium and has no relation whatever to the walls of the aquarium.

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[1] I wish to thank the research staff of the Fish Commission for placing the aquaria at my disposal.

In Fig. 1 the rectangle represents the face of the aquarium; the large arrows indicate the direction of the stimulating object, and the smaller arrows represent the fish, showing their orientation and direction of movement.

It makes no difference whether the moving object is anterior to the fish (*A*) or posterior (*B*), their orientation is the same. In the former case they move parallel too and toward the object, in the latter case away from it.

By getting above the aquarium it was determined that the fish react in the same way to objects moving in that plane.

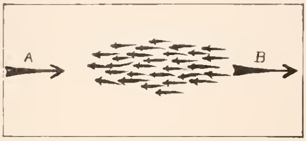

FIG. 1.

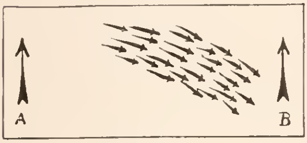

FIG. 2.

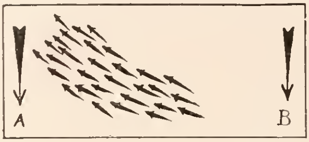

FIG. 3.

A flounder was introduced into the aquarium and swimming on the bottom beneath the sticklebacks produced the characteristic reflex.

Almost any variation of these movements may be induced by appropriate movement of the stimulating object, for example the fish can be driven from the upper right hand corner to the lower left hand corner by moving the object in the opposite direction.

By moving the object in a vertical direction the normal horizontal path can be changed up or down at will and it makes no difference whether the stimulating object is anterior or posterior to the fish. Figs. 2 and 3 show the deviation of the horizontal path.

If the stimulating object be moved slowly in this experiment the path is changed deliberately, but when the stimulus is rapid the fish may shoot in the new direction with great alacrity. A horizontal movement above the fish and at right angles to their long axes results in a deviation of their course, either to the right or to the left, according to whether the stimulating object be moved to the left or to the right respectively. Figs. 2 and 3 illustrate this feature also, if the rectangle represent the top of the aquarium instead of its face.

By using two moving objects for the purpose of stimulation the most fascinating manouvers may be elicited. A group collected at the center of the aquarium (Fig. 4, *o*) may be broken into two platoons which move toward the ends when the stimulating objects approach the center from opposite directions. These platoons may be made to turn up or down, to face about and by quickly moving the stimulating objects out from the center to charge upon one another and intermingle at the center again (Fig. 5). These reflex acts are most striking in their regularity and are executed with a military precision which never fails.

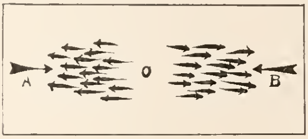

FIG. 4.

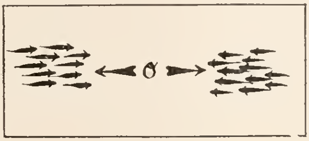

FIG. 5.

Another experiment may be performed as follows : The school of fish is brought to the center of the aquarium and the stimulating object is moved horizontally toward or away from it. In the former case some of the fish dart swiftly away to the back of the aquarium as if in fright, the others turn some to the right others to the left, thus forming two platoons as in the previous experiment, but when the object again recedes they swing about and collect in the center. In this experiment the animals which are in a direct line with the moving object are the ones to scurry away, but those outside this line are stimulated as if by some lateral change in the position of the moving body.

That there may be such an apparent lateral change can be seen by a glance at Fig. 6, in which the movement of an object from *A* to *B* has an apparent lateral motion, for example from *C* to *B* if viewed from a point outside the line of actual motion. The fish is stimulated accordingly and oriented as indicated in the diagram.

A study of the orientation of any individual fish gives the following result: When a fish is headed exactly toward the moving object a movement in any direction may be elicited, up, down, or to either side, by simply moving the object in a direction opposite to that which it is desired to make the fish take, as is indicated in Fig. 7, *a*. If the fish's long axis forms an angle with the line of motion of the stimulating object, the direction of turning in assuming its reflex orientation will depend upon which way the animal is heading. If it has somewhat the "anti"-postion with reference to the motion (Fig. 7, *b*) it will turn further into that position until its axis is parallel to the line of motion, but if it has the "homo"-position (Fig. 7, *c*) the reaction is not so certain. The fish will of course be oriented as we have already described, but it usually backs off in a hesitating way and may then turn in either direction as is indicated by the two arrows, usually however it turns in the longer arc of a circle as is indicated by the arrow at *x*, at least this is usually the case when the stimulating object is moved quickly. With a slower motion the fish usually moves through the shorter arc just as is indicated in *a* and *b*, Fig. 7.

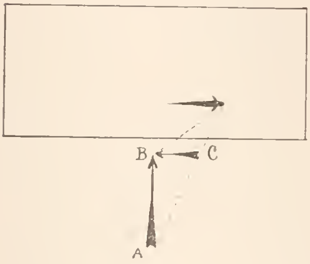

Fig. 6.

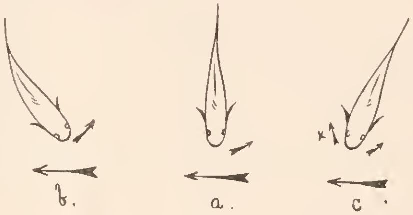

Fig. 7.

The reactions of a number of kinds of small fish were tested among them *Fundulus margalis*, *Fundulus heteroclitus*, small eels and young sea robins, but without eliciting the reflex. Young mackerel however behaved in a manner very similar to sticklebacks, but the reactions were not so precise.[1]

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[1] This reflex of sticklebacks is not invariably nor even easily obtained in small aquaria; the aquaria of the Fish Commission are about six feet long. Dr. Gaylord Clark informs me that he has tested sticklebacks for this reaction in the open water off the wharves, with positive results.

This reflex is, on the whole, most striking in its simplicity, but it is none the less puzzling for it might seem from the above experiments that the sticklebacks, and probably some other kinds of fish, form an exception to the quite general rule among animals, that by a "compensatory motion" of either the eyes, head, or whole body, the visual field is kept relatively constant.[1]

To test the validity of this view several kinds of fish were experimented with in order to determine their reactions to changes in the visual field.

1. Sticklebacks among other fish were placed in a cylindrical glass vessel which was suspended from above. Beneath and fitting quite closely about this aquarium was placed a cylindrical galvanized iron dish resting on a turn-table. The interior of this iron dish was striped alternately black and white. When this visual field was rotated the fish were very definitely oriented and swam with it, although the aquarium and water remained stationary. The fish made the compensatory effort to keep the visual field constant.

2. The animals were placed in the same cylindrical aquarium and the water set in motion by a gentle circular motion of the hand the idea being to simulate the classic turn-table experiments. As a result the animals headed up stream (rheotropism). Their efforts were materially increased by rotating the visual field opposite to the moving water, or they could be brought to rest so that they floated with the stream by rotating the field at about the speed of the moving water and in the same direction. Dr. Lyon was at the same time conducting experiments on rheotropism and by several most ingenious methods demonstrated an orientation where no current existed and where the reaction was produced only by a changing visual field.[2] Most of the fish he tested showed the same tendency to keep a constant visual field.

The results of these experiments seem at variance with those set forth in the first part of this paper, but there is this difference to be noted. The reflex depends upon a stationary visual field with the stimulating object moving before this background, a fact which offers a possible explanation of the peculiar reflex. If we fix the eyes on any near object which is then moved, the whole visual field has an apparent motion in the opposite sense. Seated in a moving car our eyes follow the near objects as they are passed, but the *distant* landscape appears to revolve in a direction *with* the moving train. As a tentative hypothesis it may be assumed that in our experiments the moving stimulating object fixes the attention of the fish, the apparent motion of the visual field as a whole is then in a direction opposite to that of the moving object. This apparent motion may be the determining factor in causing the orientation and movement which is the essence of this peculiar reflex shown by sticklebacks.

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[1] E. P. Lyon, *American Journal of Physiology*, 1899, III.

[2] E. P. Lyon, *American Journal of Physiology*, 1904, XII., p. 153.

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THE PHYSIOLOGY OF LOCOMOTION IN GASTEROPODS.

A. J. CARLSON.

While studying the physiology of the molluscan heart I incidentally observed in the snail a mode of locomotion which would seem to explain the mechanism by which the series of waves of contraction and relaxation of the sole of the gasteropod foot in locomotion are produced. The musculature of the foot and the ordinary movements of locomotion of land gasteropods by a series of alternating contractions and expansions, passing as waves over the sole of the foot in the postero-anterior direction, has been described by Simroth (1878, 1879).[1] Simroth's observations were made principally on *Helix* and *Limax*. In attempting to correlate the form and sequence of these waves of locomotion with the structure of the musculature of the foot Simroth concludes that they cannot be produced by the separate or combined contractions of the oblique and the transverse muscular strands. The cause of the extension of the foot is to be sought in the active extension of the longitudinal musculature; that is, when the muscle-cells making up these strands contract they elongate and decrease in thickness. These he therefore calls "extensile muskulatur" in contradistinction from the oblique and the transverse muscle, which is of the ordinary contractile type.

This theory of "extensile muskulatur" may explain the series of elongations of the foot in locomotion, but the question is, Is it true? The theory may without much difficulty be put to the experimental test by any one interested. Simroth does not show that the muscle-cells making up the longitudinal musculature of the foot increase in length and decrease in diameter on direct stimulation or on stimulation of the pedal nerves. A few ex-

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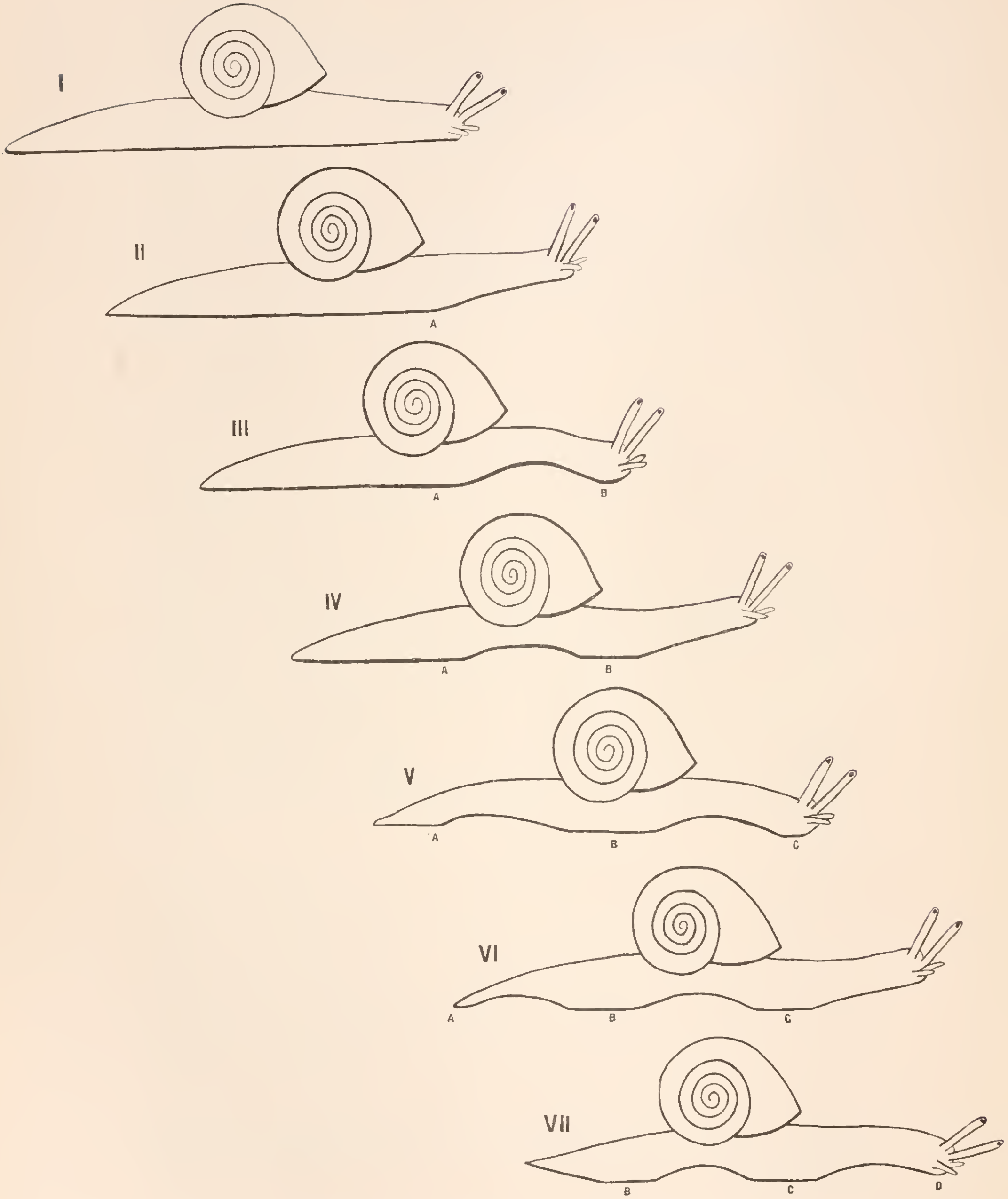
[1] Simroth, H., "Die Thätigkeit der willkürlichen Muskulatur unserer Landsnecken," *Zeitschr. f. wiss. Zoöl.*, XXX., p. 166; "Die Bewegung unserer Landsnecken, hauptsächlich erörtert an der Sohle des Limax," *Zeitschr. f. wiss. Zoöl.*, XXXII., p. 284.


I
II
A
III
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VI
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VII
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D

Locomotion of the snail. For description see text.

periments in that line would have convinced him of the fallacy of his theory. I have tested the longitudinal muscle of the foot in several gasteropods and my results go to show that there is no difference between the physiology of this muscle and that of any other muscle. On stimulation, either directly or through the motor nerves, the muscle-cells or strands of muscle-cells shorten and thicken in the usual way. These experiments may be performed with the greatest ease on the foot of *Pleurobranchæa*, as the foot musculature of this gasteropod is composed of a very loose meshwork of septa that may be separated the one from the other without sufficient injury to produce extreme contraction. In gasteropods with very compact foot this cannot be done, as the injury of dissection produces extreme contraction. But even when the foot musculature is greatly contracted direct stimulation with the induced electrical current produces, not an elongation, but a further shortening and thickening of the muscle. In no case does the stimulation produce elongation of the longitudinal muscle strands.

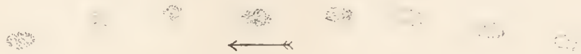

FIG. 1. Tracings, ½ natural size, of the tracks of a snail moving in the manner illustrated on previous page. The dotted areas are the areas covered by the mucus film. The arrow indicates direction of the motion. Medium-sized animal, moving rapidly.

Jordan (1901)[1] rejects the theory of "extensile muskulatur" in accounting for the locomotion in the marine gasteropod *Aplysia*, and ascribes the relaxation or extension of the longitudinal muscle of the foot to the pressure of isolated bodies of the visceral fluid or blood. As evidence Jordan points to small reservoirs or lakelets of plasma in the strongly contracted foot. These lakelets are constricted off from the visceral cavity by the contraction of the muscular septa. A body of liquid thus cut off from the visceral cavity may serve to produce extension of the longitudinal muscle at its anterior border by the force of contraction of the oblique and transverse muscles at its posterior end. In this way we would have as many isolated bodies of blood being gradually pushed from behind forwards in the foot as there are areas of relaxation on the sole of the foot. The presence of isolated bodies of liquid in the strongly contracted foot is not a sufficient evidence that they are the factors in producing the waves of locomotion, as similar isolated bodies of liquid are also found in the musculature of the contracted mantle (*Aplysia*, *Pleurobranchæa*).

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[1] Jordan, H., "Die Physiologie der Locomotion bei Aplysia limacina," *Zeitschr. f. Biologie*, XLI., p. 196.

The arrangement of the muscle in the foot and the appearance of the sole of the foot in locomotion are so similar in all gasteropods that the mechanism by which locomotion is effected is in all probability the same in all. Simroth and Jordan missed the true explanation by not taking into account the part played by the musculature of the dorsal and lateral walls of the body cavity.

The mode of progression of the snail which furnishes the key to the solution is represented in the series of diagrams on page 2. Diagram I. represents the side view of the snail during ordinary locomotion. The edges of the foot touches the ground throughout the whole length of the animal, and a continuous trail of mucus marks the path of progression. When the animal changes this gait to that illustrated in diagrams II. to VII. the head is lifted from the ground and while pushed forward by the progression of the rest of the body the neck or successive portions of the anterior end is elongated and the diameter diminished as it leaves the contact with the ground. In a few seconds the anterior third of the animal comes to assume the position shown in diagram II. This elongated head end is being held clear of the ground at an angle of 20 to 30 degrees. All this while the animal progresses by means of the part of the foot still in contact with the ground. When the anterior one fourth or one third of the body has attained position II., the head bends down so that the very anterior end of the foot again comes in contact with the ground. The neck and anterior part of the body are bent to form an arch in the manner shown in diagram III. The distance from the ground to the highest point of the curve is from 2–4 mm. The posterior end of the body now appears literally to flow through this arch to the new point of contact (*b*), that is, as the posterior part of the body moves forward the successive portions form their respective parts of the curve. The space of ground between *a* and *b* is not touched by the foot in any region of the body. While the middle third of the body is thus pushed and pulled forward, elongating, diminishing in diameter and bending away from the ground to form the arch *a–b* (diagram IV.), the head end repeats the performance of diagram II. The foot at the head end continues in contact with the ground only for a distance of one to one and a half centimeter. When the head and neck bend away from the ground, the neck elongates as before, and we have the anterior third of the body in diagram IV. in a position similar to that in diagram II., the head ready to bend down to make a new contact (*c*). The next stage is shown in diagram V. The middle third of the body has advanced to the second point of contact (*b*) and the arch stage or state of elongation and decrease in diameter is being assumed by the last third. The head end repeats the performance of diagram II. and IV., till in VI. it is again elongated and raised from the ground. The snail has now traversed a distance equal to the length of its own body, so that only the extreme tail end of the foot touches the original contact (*a*). The tail end is very much reduced in diameter at this stage. As the head bends down to make the fourth contact (*d*) the tail end is being lifted high from the ground and pulled up to contact *b* in the manner shown in diagram VII. Further progression is simply a repetition of these phenomena. When the whole body has come into this mode of progression the foot is thus in contact with the ground

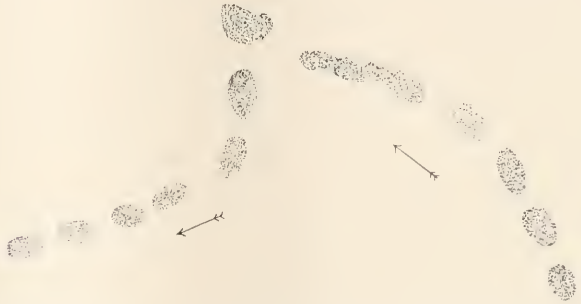

FIG. 2. Same as Fig. 1. Portions of the track of a large specimen moving slowly. ½ natural size.

only at three points, the intervening parts being held in the position of arches clear of the ground. In some instances the foot may be in contact with the ground only at two places, as in diagram VI. the tail end may be lifted from the ground before the head bends down to make the next contact. At any one time there will thus be two regions of elongation and decrease in diameter of the body and three regions of shortening and thickening of the body and *vice versa*.

When moving in this manner the snail does not leave a continuous film of mucus along its path as in ordinary locomotion, but a trail like the ones represented in Figs. 1 and 2 on page 2. These figures are traced from the tracks made by the snails across the table, the surface of which was covered with a thin layer of dust. The arrows indicate the direction of the motion, the dotted areas are the places touched by the foot and hence covered with a film of mucus. Fig. 1 is a portion of the track of a snail of medium size moving straight ahead at a rapid rate. The areas of contact of the foot with the surface are smaller and the further apart the quicker the progression. Fig. 2 is taken from the trail of a larger snail moving slowly and not in a straight line. The areas of contact are larger and closer together, and may even fuse.

This mode of locomotion enables the animal to cover the ground much quicker than in the ordinary way, and it was always resorted to by the snail studied (*Helix dupetithouarsi*) when endeavoring to escape from an enemy. I observed it for the first time after having punctured the apex of the shell preparatory to injection of alkaloids into the animal. When replaced on the table the snail quickly emerged from the shell and started this gallop across the table. But the snail will make use of this mode of locomotion when not in the least injured. For some reason I never succeeded in making a specimen move in this manner across a surface covered with lampblack. If a smoked paper was placed in the path of a galloping snail the animal resorted to the ordinary locomotion on touching it. I have not observed this mode of locomotion in the slugs (*Limax*, *Ariolimax*) or in any of the marine gasteropods.

What is the mechanism of this mode of progression and in what way does it differ from the ordinary locomotion? Referring again to the diagrams on page 2 it is obvious that the series of changes of form and position of the anterior third of the body resulting in the position shown in diagram II. cannot be brought about by the musculature of the foot alone, even if aided by isolated bodies of liquid. The entire musculature of this end of the body must be brought into play. If the transverse and the oblique muscular strands contract gradually at successive levels from behind forwards simultaneous with the relaxation of the longitudinal muscle, this part of body would elongate and decrease in diameter just as actually occurs. The elevated position of the elongated head end is simply due to the greater relaxation of the longitudinal muscle in the foot than in the dorsum. The pushing forward of the head end is probably aided by the pressure of the viscera.

The bending down (or in any direction) of the head end is obviously a result of the contraction of the longitudinal muscle in the foot simutaneous with the relaxation of that in the dorsum. If we assume that the posterior half of the elongated head end retains the original contraction of the several muscle systems and that in the anterior half the foot contracts and the dorsum relaxes, we have the downward movement of the head and the formation of the arch. The passage of the middle third of the body through the ascending limb of the arch is simply the continuation of the processes of contraction of the transverse and oblique muscle and relaxation of the longitudinal muscle which brought about the elongation and elevation of the head end. The same muscular mechanisms which must act in the latter case suffice to account for the former. In the descending limb of the arch the relation of contraction and relaxation of the systems of muscles is reversed. The transverse and the oblique muscles relax and the longitudinal muscles contract, pulling that part of the body forward and down to point *b* (diagram II.). Friction is probably sufficient to prevent the head end being pulled backward instead of the body forward by this contraction. The lifting up and subsequent shortening and thickening of the tail end in diagrams VI. and VII. is simply a relatively sudden contraction of the dorsum of this part and subsequent contraction of all the longitudinal muscle strands. By thus taking into account the entire body musculature, their coördinate contraction and relaxation suffice to account for these changes in form and position without having recourse to any " extensile muskulatur " or series of isolated bodies of liquid in the foot.

What has this mode of progression in common with the ordinary locomotion? In the chitons the dorsal shells prevent any considerable contraction and elongation of the dorsum. And even in ordinary locomotion of other marine gasteropods as well as of the pulmonates there is no appreciable elongation and shortening of the dorsum corresponding to the waves of locomotion on the sole of the foot. Nevertheless the peculiar mode of progression in the snail just described is probably only an exaggerated form of the ordinary locomotion. During ordinary progression the animal assumes its greatest length and smallest diameter; to account for this we need nothing further than the contraction of the transverse and the oblique muscles of the dorsal and lateral sides of the body. The waves of locomotion in the foot are diminutive representatives of the waves of relaxation and contraction illustrated in the diagrams on page 2. At the areas of relaxation the sole of the foot adheres closely to the ground, and between these points the sole is slightly elevated. Nevertheless a continuous layer of mucus covers the path of progress, as the areas of contact are close together and subsequent portions of the foot occupying the same area is pulled forwards a little so as to finally touch the preceding area of contact. There can be little doubt that the area of contact of the foot with the ground in any region serves as a fixed point through friction and acting on this the contraction of the longitudinal muscles of the foot pulls the neighboring portion of the body forwards.

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FORM-REGULATION IN CERIANTHUS, VIII.

Supplementary Partial Discs and Heteromorphic Tentacles.

C. M. CHILD.

The Regeneration of Supplementary Partial Discs and Correlated Regulation.

The regeneration of supplementary partial discs and tentacles in *Cerianthus* from the lateral body-wall was first described by Loeb ('91). The method of procedure employed in order to produce results of this kind was partial transverse section of the body-wall at one or more levels. According to Loeb it is necessary to delay the healing of the wound for a few days in order to bring about tentacle regeneration in connection with it. The regeneration of tentacles and disc always occurs in connection with the lower (aboral) side of the wound. The number of tentacles borne by the supplementary disc is a fraction of the whole number characteristic of the complete disc and varies with the portion of the circumference involved in the cut. When the cut occurs near the oral end of the body the supplementary disc possesses a mouth, but discs regenerated at a lower level, *i. e.*, further aborally, possess no mouth.

Loeb regarded these experiments as affording an important indication of the relation of growth to turgor of the cells. After a transverse cut is made in one side of the body-wall the tentacles oral to this region "lose their turgor" while the other tentacles retain theirs. This fact, as Loeb believes, indicates that the distension or erection of the tentacles cannot be due to water under pressure in the enteric cavity since if that were the case an opening into the enteron would cause the collapse of all tentacles, and he concludes that the cut has in some way reduced the turgor of the cells, comparing the effect with the withering of plant tissues after the stem is cut. No suggestion as to the manner in which the cut effects loss of turgor in the cells of the tentacles is given. Certainly the case is not comparable with that of the plant for the tissues of *Cerianthus* are bathed internally and externally by water and it is very difficult to see how or why a cut on one side of the body at a distance of two to three centimeters from the tentacles should cause loss of turgor in those tentacles directly above it. There are no vessels or tubes of any kind running longitudinally in the body-wall. If the shock or irritation resulting from the cut is the cause of the loss of turgor why should it be transmitted only in the oral direction?

Apparently Loeb still holds the view originally expressed regarding these phenomena for he has recently called attention to these experiments and to his conclusions (Loeb, '03). As a matter of fact, however, the regeneration of these supplementary partial discs and tentacles and the reduction of the tentacles oral to them constitute most striking evidence in favor of the view that internal, *i. e.*, enteric, water-pressure is an important regulative factor in *Cerianthus.* There is not the slightest ground in support of the conclusion that the direct effect of a cut upon intracellular turgor extends beyond the cells in the immediate vicinity of the injury.

In my own experiments both *C. membranaceus* and *C. solitarius* were used, the latter species chiefly. Similar results were obtained from both species, as in the case of other regulative phenomena. Description of a few of my experiments and a brief analysis of the results obtained will show very clearly that the supplementary partial discs and tentacles regenerate in exactly the same manner as typical localized structures, and that reduction of tentacles above the accessory discs is due, like other cases of tentacle-reduction described, to decrease in internal pressure.

The experiments group themselves under several categories: the results differ somewhat according as the operation is within the œsophageal region or aboral to it; moreover, the effects of these operations both upon regeneration of tentacles and upon their reduction must be considered. I shall discuss first the regulation of lateral openings in the œsophageal region in relation to tentacle regeneration, and tentacle reduction, and then the regulation of lateral openings in regions of the body aboral to the œsophagus in relation to tentacle-regeneration and reduction.

Supplementary Discs in the Œsophageal Region.

The method employed in producing partial supplementary discs in the œsophageal region was as follows: a transverse cut was made in one sideof the body-wall near the middle of the œsophageal region (Fig. 1), thus interrupting on one side of the body the continuity of both œsophagus and body-wall, while on the opposite side both remained intact. Loeb removed small pieces of the body-wall near the middle of the œsophageal region in his experiments of this kind, thus preventing rapid healing of the cut edges (Loeb, '91, text fig. 1). I found, however, that this was not necessary in the œsophageal region, the mere transverse slit being in most cases sufficient to bring about the desired results. Neither was it necessary to delay or prevent the union of the margins of the body-wall by artificial means such as the wire netting employed by Loeb for this purpose.[1]

In my experiments the pieces were usually left undisturbed after the operation, though occasionally it was necessary to cut a piece a second time in order to cause the production of a supplementary disc. Loeb ('91, p. 56) himself noted that the cut showed a much stronger tendency to remain open when situated near the oral end, but apparently was unaware that this was due to the presence of the œsophagus.

The history of tentacle regeneration in a piece of this kind is given as an example.

Series 42.

October 24, 1902. — A large specimen (*C. solitarius*) of normal appearance was subjected to the operation indicated in Fig. 1. First disc and tentacles were removed by a transverse cut just aboral to the disc. Then a transverse cut extending about half way through both body and œsophagus was made near the middle of the œsophageal region. After section the piece collapsed, as in all cases where the enteron is opened, and became distended only when closure of the enteron occurred, either by union of cut surfaces or by approximation.

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[1] The meshes of this netting were somewhat smaller than the diameter of the body and the animals, being laid upon the netting, pushed their bodies through it, aboral ends first. until the level of the partial transverse cut was reached. The wires being forced into the cut prevented further movement and also, according to Loeb, delayed union of the two cut surfaces.

November 1. — Eight days after section. On the oral end of the piece the œsophagus and body-wall have united about the whole circumference of the body, the usual result in such cases ; the free surfaces of the body-wall formed by the transverse cut have not united with each other, but each has united with the corresponding cut œsophageal surface. Fig. 2,[1] a diagrammatic longitudinal section of the body at this stage, shows what has occurred more clearly than is possible by description. It is evident that that part of the body directly over (*i. e.*, oral to) the lateral cut has closed in such a manner that its enteric cavity is not in communication with that of other parts of the body nor with the exterior. Orally and aborally it is closed by the union of œsophagus and body-wall ; each intermesenterial chamber is separated from adjoining chambers by the intervening mesentery. Thus, as regards this region, conditions are similar to those ex-

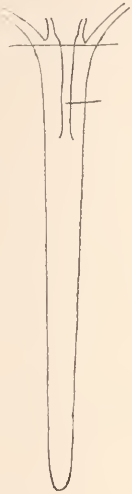

FIG. 1.

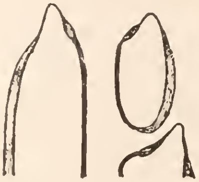

FIG. 2.

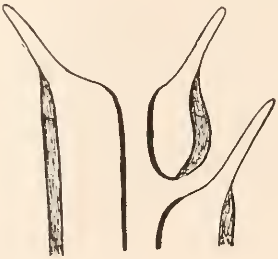

FIG. 3.

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[1] The different regions are distinguished in these figures in a somewhat conventional manner. The old body-wall is represented by two heavy lines (ectoderm and entoderm) with fine longitudinal lines indicating the longitudinal muscles between them. The œsophageal region is represented by a very heavy single line and regenerated regions by a much lighter single line. In Figs. 7 and 8 portions of old disc and tentacles are represented by a double line and regenerated disc and tentacles by a single line.

isting in the œsophageal pieces described in a previous paper (Child, '04*d*). Consequently water can enter this portion only by diffusion or as a secretion.

The lower (*i. e.*, aboral) margin of the transverse cut and the oral end of the body on the uncut side present similar conditions (Fig. 2). In both regions the union of œsophagus and body-wall produces conditions apparently favorable for typical tentacle regeneration ; in both the intermesenterial chambers are in free communication with the main enteric cavity and distension and free movement of the circulatory currents is therefore possible.

It is clear from the diagram (Fig. 2) that each of the discs will form a mouth. The relations of these two mouths is also sufficiently evident from the figure.

If the conclusions reached in preceding papers ('04*b*, '04*c*, '04*d*) are valid we may expect that tentacle-regeneration will occur in the typical manner on the oral margins of the body of the uncut side and on the aboral margin of the transverse cut, *i. e.*, the lower margin which corresponds to an oral end with respect to that part of the body aboral to it.

On the oral end of the body over the transverse cut regeneration must be delayed if internal water-pressure is effective, since only the slight distension due to diffusion or secretion is possible in this region. The history of the piece fulfills all expectations.

For convenience the regenerating region at the oral end of the piece may be designated as terminal and that on the side of the body as lateral. It is also necessary to distinguish between the cut side of the body, that on which the lateral cut is made and the uncut side.

Regeneration of the marginal tentacles has already begun in this piece (Fig. 2) ; the terminal marginal tentacles on the uncut side are 1 mm. in length, those on the cut side are minute buds 0.25–0.5 mm. in length. The lateral marginal tentacles are about 0.5 mm. in length. In appearance they are more advanced than the terminal tentacles on the cut side but somewhat less advanced than those on the uncut side. The portion of the body between the lateral cut and the oral end is very evidently less distended than the other parts.

November 6. — Thirteen days after section. Terminal marginal tentacles on uncut side 3 mm., on cut side 2–2.5 mm.; lateral marginal tentacles 3 mm. The regeneration of the tentacles over the lateral cut is somewhat retarded. Fig. 3 represents a diagrammatic longitudinal section through the œsophageal region at this stage.

November 12. — Nineteen days after section (Figs. 4 and 5). Terminal marginal tentacles on uncut side 6–7 mm.; on cut side 1 mm.: lateral marginal tentacles 6–7 mm. Terminal labial tentacles on uncut side 1 mm.; on cut side absent: lateral labial tentacles 1 mm. The terminal disc on the uncut side and the partial lateral disc are well distended, while that part of the terminal disc which lies over the cut is collapsed and wrinkled. The distended portion of the lateral disc and the terminal disc are gradually approaching the same level. At this stage the regeneration of the terminal tentacles over the cut is not only retarded but has ceased and the tentacles are decreasing in size. The wrinkled appearance of the portion of the body bearing these tentacles shows clearly that it does not share in the distension of the other parts.

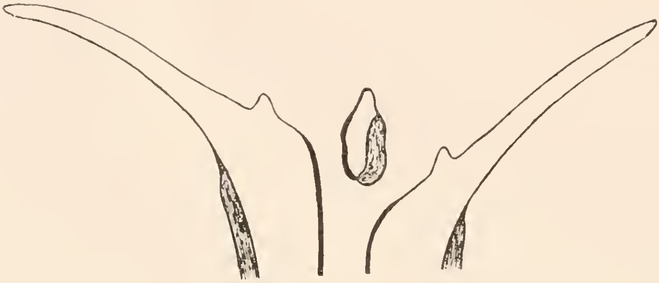

FIG. 4.

The diagrammatic figures (Figs. 4 and 5) represent respectively the relations of parts in longitudinal section and in oral aspect. In Fig. 5 the shrivelled half of the margin with its reduced tentacles is clearly shown. One tentacle at each end of this reduced region is somewhat longer than the other reduced tentacles. Evidently this tentacle on each side arises over the intermesenterial chamber abjoining the distended part of the body. Probably the greater length of these two as compared with the other reduced tentacles is due to the greater degree of distension of the intermesenterial chambers below them. It may be that these chambers are not completely shut off from the enteric cavity or possibly the pressure of the water in the distended region causes filtration of water into these two chambers. Figs. 4 and 5 illustrate very clearly the relation between characteristic form and internal water pressure.

November 20. — Twenty seven days after section (Fig. 6).

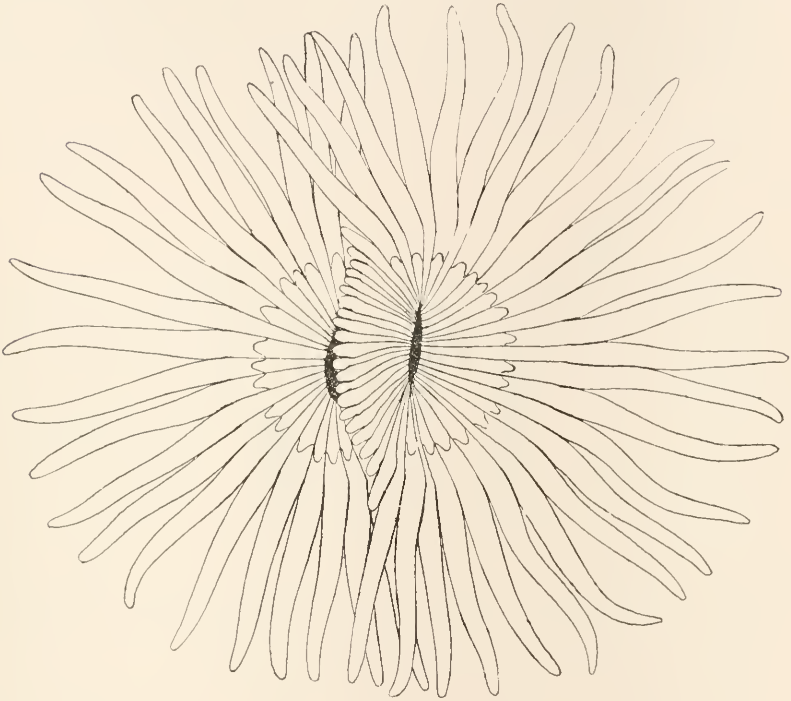

Fig. 5.

Terminal marginal tentacles on uncut side 8–9 mm.; on cut side barely visible 0.25 mm.: lateral marginal tentacles 8–9 mm. Terminal labial tentacles on uncut side 2 mm., on cut side absent: lateral labial tentacles 2 mm. The portion above the lateral cut is much shrivelled while all other parts are fully distended and in good condition. The lateral and terminal discs are now at almost the same level (Fig. 6), the part which originally formed one side of the terminal disc being now represented only by the narrow shrivelled strip separating the two discs. About thirty days after section the shrivelled portion separating the two discs was ruptured by the increase in diameter of the discs. Each portion of it remained attached to the disc on its own side but gradually underwent atrophy. Reference to Fig. 6 renders it evident that this shrivelled strip was all that separated the two mouths: with its rupture the two mouths became one. The two discs, or two parts of the disc continued to approach the same level, the constriction marking the region where the shrivelled strip stretched across (Fig. 5) gradually disappeared and on December 12, forty-nine days after section the specimen was normal in appearance, with marginal tentacles about 12 mm. in length and labial tentacles about 3 mm.

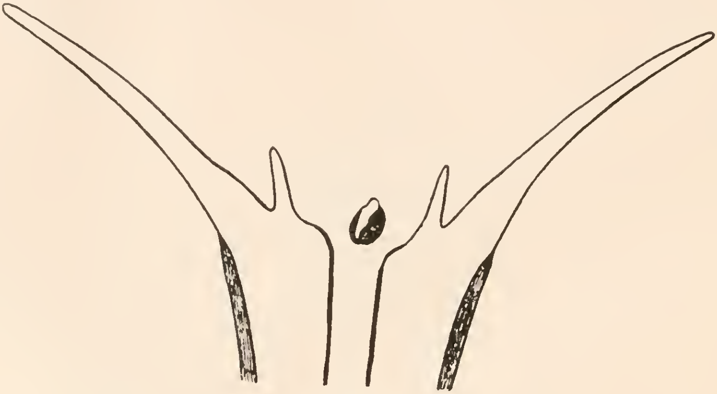

Fig. 6.

The history of other specimens of the same sort corresponds closely with that of the piece just described. It is possible of course to modify the results in various ways: for example the lateral cut may be made more or less deep and thus cause the isolation of a greater or less number of intermesenterial chambers above it and consequently the retardation in regeneration and the later reduction of a larger or smaller number of tentacles. It is possible to make the cut so deep that only three or four tentacles remain distended on the terminal disc all the rest appearing on the lateral disc. On the other hand, if the cut involves only a small portion of the œsophageal circumference, only a few tentacles are retarded and correspondingly only a few appear on the lateral disc. If the cut is not deep enough to reach the œsophagus, but involves only the body-wall, the cut surfaces of the body-wall unite within a few days and regeneration of the terminal disc proceeds in the typical manner.

Turning now to the consideration of specimens with fully developed normal tentacles, the effect of a lateral cut in the œsophageal region is not difficult to understand if we recall the reduction and atrophy of tentacles in consequence of reduced internal pressure which was discussed in the preceding paper (Child, '04*e*), and if we bear in mind also the method of closure in series 42. For comparison with series 42 the history of a piece of this kind is given briefly.

Series 10.

September 12, 1902. — A specimen with marginal tentacles about 25 mm. and labial tentacles about 8–9 mm. in length was used. A lateral transverse cut extending about half way through the body was made in the œsophageal region as in Series 42 (Fig. 1) but in the present case the original disc and tentacles were not removed as in Series 42.

September 19. — Seven days after section. Closure has occurred as shown in Fig. 7 and the specimen is distended. The tentacles on the uncut side retain their original length; marginal 25 mm., labial 8 mm.; those on the cut side are reduced — marginals 15–18 mm., labials 6 mm. The portion above the lateral cut in which, as in Series 42, the intermesenterial chambers are completely shut off from the exterior and from the main enteric cavity, is less distended than the other portions as indicated in Fig. 7. In this figure only the bases of the old tentacles are shown but the smaller size of those on the right is indicated.

The cut surfaces of œsophagus and body-wall on the lower (aboral) side of the cut have united as in Series 42 and now the regenerating marginal tentacles are about 2 mm. in length on this supplementary disc (Fig. 7).

October 3. — Twenty-one days after section (Fig. 8). The tentacles on the uncut side still retain nearly their original length

— marginal 20–22 mm., labial 7–8 mm. The tentacles oral to the lateral cut are now greatly reduced — marginal 3–4 mm., labial 1–2 mm. — and the tips are shrivelled; the whole region above the lateral cut is greatly shrunken and wrinkled, containing almost no water (Fig. 8).

Aboral to the cut the supplementary tentacles have regenerated in the typical manner — marginals 10 mm., labials 3–4 mm. The new supplementary disc on the right, and the left half of the old disc now lie at nearly the same level.

A few days later the shrivelled strip bearing the reduced tentacles was ruptured and a part dropped off. The remaining portions underwent complete resorption within a few days, and a disc of typical form resulted. One half of this disc represented what remained of the old disc and its tentacles were the tentacles of the original specimen; the other half was the regenerated supplementary disc. The tentacles on the old portion were still considerably longer than on the regenerated portion, but a gradual equalization in length occurred as might be expected since conditions of internal pressure are similar about the whole circumference.

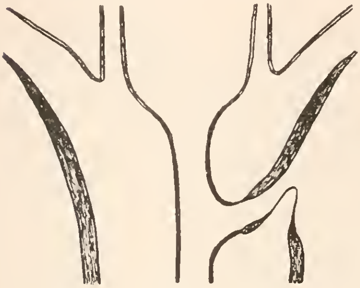

FIG. 7.

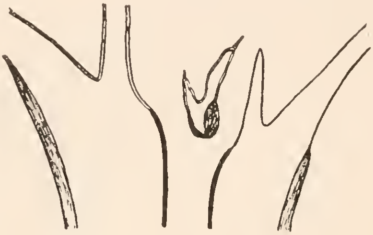

FIG. 8.

The history of other similar specimens is similar in all essential respects to that described, though the number of tentacles which undergo atrophy vary according to the size of the lateral cut. It is possible to modify these experiments in many ways but in every case that part which has no communication with the main enteric cavity or the exterior and so does not remain distended undergoes atrophy, while those regions in which internal pressure persists either retain their form and size or regenerate as the case may be.

The atrophy of the tentacles above the cut in series 10 is very clearly due to the same factor which caused retardation in regeneration and later reduction and atrophy of the tentacles over the cut in series 42. In both cases the conditions in the strip above the lateral cut are exactly similar to those which exist in œsophageal pieces (Child, '04*d*). Comparison of the history of these strips with that of the œsophageal pieces will show that both are affected in exactly the same way. Some degree of internal pressure may be established by osmosis or secretion after closure, but this gradually diminishes, the tentacles atrophy, and the whole piece shrivels and finally breaks up.

The relative change in level of the two partial discs, which results in the production of a disc of typical form and in typical position with respect to the body axes, is a features of special interest. It is impossible to determine from the appearance after regulation is completed whether the lateral supplementary disc moves orally to the level of the old disc, or *vice versa*, or finally whether both change their level, the old partial disc moving aborally and the new orally until they attain approximately the same level.

There is always in a regenerating end a certain amount of growth in the oral direction due undoubtedly to internal pressure. The supplementary partial disc changes its level in the oral direction in consequence of growth. This change brings it nearer to the level of the old disc, which of course has not been altered since its tissues are fully differentiated and not in a condition for rapid growth. Probably this oral movement of the regenerating disc is sufficient, when it is situated in the œsophageal region, to bring it approximately to the same level as the old disc. If differences in level remain after the regenerative growth is completed, I think it is probable that further regulation may occur in the manner discussed in connection with oblique pieces (Child, '04*d*), viz., through the attempt of the animal to orient disc and body-axis in a definite manner. In the pieces with supplementary partial discs in the œsophageal region the difference of level between the supplementary disc and the old disc is greater during the contracted condition than during distension, as in pieces with oblique discs. This fact indicates that the muscles of the body-wall below the supplementary disc are more completely relaxed during distension and orientation than those of the opposite side (Child, '04*d*). The tissues gradually adapt themselves to the altered conditions and the final result is the form in which orientation in the typical manner is possible, *i. e.*, the typical or normal form.

Supplementary Partial Discs Aboral to the Œsophagus.

Loeb ('91) found it possible to produce the partial discs at any level of the body except the extreme aboral region, but stated that the tendency to closure of the cut without the formation of a new disc is greater in the middle region than near the oral end. Moreover, he found that the partial discs produced in the middle region were without mouth-openings.

My results agree with Loeb's in regard to these points, but, since he did not turn his attention to the internal anatomy nor investigate the nature of the distension, the reasons for these differences did not become apparent to him. Apparently he was not even aware that in making the lateral cut near the oral end he was cutting into the œsophagus, while farther aborally only the general enteric cavity was opened. I desire, therefore, to point out certain very definite reasons why the results should differ according to the position of the lateral cut in the œsophageal region or aboral to it.

There are several points to be considered, but the question as to the effect of the lateral cut on the tentacles directly oral to it naturally takes precedence as one of the immediate consequences of the cut.

According to Loeb, a lateral cut in the middle or aboral region of the body does not cause any permanent difference between the tentacles directly oral to it and the others; here also my results agree with his. His Figs. 2 and 3 show cases of this kind in which the tentacles at the oral end are equal in length about the whole circumference.

If the collapse and reduction of tentacles be due to loss of intracellular turgor in consequence of the cut there is no reason apparent for the permanent reduction of the tentacles after a cut near the oral end and the absence of any such effect after a cut near the middle. The difference in the distance between cut and tentacles in the two cases is certainly not sufficient to justify the conclusion that in the one case a shock or stimulus of some sort reaches the tentacles while in the other case it does not, nor can it serve as a basis for explanation if the change be regarded as simply osmotic in nature, not as a reaction to a stimulus.

If, however, we consider the difference in the relations of parts in the two regions of the body and the effect upon the internal pressure all difficulty disappears. If the lateral cut be made at any of the levels indicated in Fig. 9 it will simply enter the intermesenterial divisions of the enteron which open axially into the central cavity everywhere aboral to the œsophagus, since the axial margins of the mesenteries hang free in the cavity. There is nothing with which either margin of the cut can unite except the other margin. When cuts at these levels close they can close only by means of union of the cut edges. It follows that the same relations between the part oral to the cut and the other regions of the body are not altered by the cut.

The immediate consequence of such a cut is loss of water from the enteron and complete collapse of body and all tentacles. Inrolling of the body-wall adjoining the cut soon begins and approximation of the inrolled portions is brought about by the elasticity of the other parts of the body-wall — especially that of the opposite side of the body (Child, '04*a*, Figs. 8 and 9). Occasionally when the cut is not far below the aboral end of the œsophagus, the contraction of the body-wall and of the thickened mesenterial margins and filaments in consequence of the cut almost or quite closes the intermesenterial chambers between the cut and the aboral end of the œsophagus. It follows that when water again enters the enteron through the œsophagus other regions will become distended, but these will not and consequently the corresponding tentacles will remain collapsed. At this stage the internal pressure is always much lower than that in normal animals since increase of the pressure beyond a certain point causes the approximated margins of the cut to separate and the water is lost. The point to which I wish to call attention, however, is this: when the lateral cut is only slightly aboral to the œsophagus the intermesenterial chambers oral to the cut may be closed temporarily and so the tentacles corresponding to them may remain less distended than the others as long as this condition continues. This difference is due simply to mechanical plugging of the openings of the intermesenterial chambers at the aboral end of the œsophagus and the consequent exclusion ot water. As soon as the cut is actually closed by new tissue the increasing distension causes stretching of the body-wall and mesenteries and the closure of the chambers can persist no longer. The tentacles in this region now become as fully distended as the others and since the period of their collapse is usually short in such cases, are soon equal in length to the others. If the intermesenterial chambers remain contracted and plugged during a long time considerable reduction of the corresponding tentacles may occur. Cases of this kind have occurred frequently among my specimens and it was always evident that the contracted condition of parts about the cut and especially oral to it was responsible for the absence of distension in tentacles oral to this region. If the collapse were osmotic in nature, as Loeb believes, there is no reason apparent why it should be permanent when the cut is in the œsophageal region and only temporary when the cut is a few millimeters lower.

Loeb found that lateral cuts in the middle and aboral regions showed a much greater tendency to close by union of their margins than those near the oral end. He does not attempt to account for this difference in any way, but the reason is evident at once when the part played by the œsophagus is understood. In the œsophageal region the cut margins of œsophagus and body-wall almost always come into contact in consequence of the inrolling (Fig. 2). The distension following union of the parts prevents the two cut surfaces of the body-wall from coming into contact with each other and, therefore, they never unite and an opening remains which is in reality a new mouth. If they happen to come into contact with each other instead of with œsophageal cut edges they unite but the usual result is determined by the fact that conditions are much more favorable for the union of body-wall and œsophagus than for body-wall and body-wall.

On the other hand, if the cut is below the œsophagus the only possible union is body-wall and body-wall (Fig. 10). If the animal is left undisturbed the cut will close in the typical manner, and as soon as distension occurs the relations of parts are once more normal in all essential respects (Fig. 11). Both Loeb and myself agree that under these circumstances a formation of a supplementary disc is not possible.

It now remains to consider how conditions are altered if the animal is inserted into a mesh of the netting (see above, p. 95),

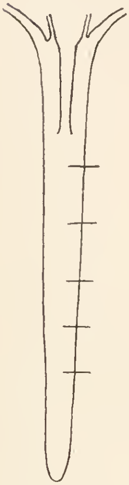
Fig 9.

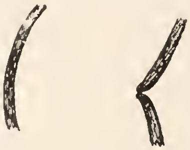
Fig. 10.

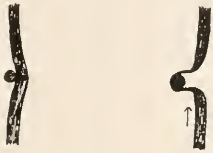
Fig. 13.

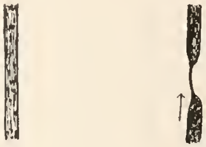
Fig. 11.

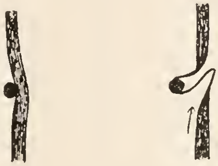
Fig. 14.

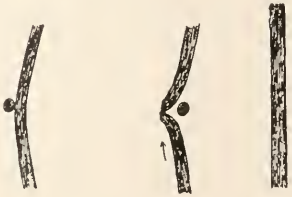
Fig. 12.

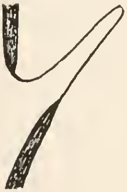
Fig. 15.

used by Loeb to cause the appearance of the supplementary disc. In Figs. 12, 13 and 14 the result of using the netting is shown. As long as the body is collapsed the netting has little effect (Fig. 12). Indeed, in my experience collapsed specimens react much less readily than others, and orientation in the netting rarely occurs until the animal is at least partly distended. It follows from this that the formation of supplementary discs after use of the netting is not necessarily due to delay in closure. As distension with water gradually occurs after closure the diameter of the body increases, but the wires of the netting prevent increase in diameter in the region of the cut. The result is indicated by Fig. 13. The thin, delicate new tissue is chiefly affected, being stretched in the direction of the tension, or more probably undergoing rapid growth in response to it. Thus a deep groove is formed on one side of the body and on the aboral side of this groove the new tentacles of the supplementary disc appear (Fig. 14).

Apparently the netting has merely prevented the body-wall from assuming the form shown in Fig. 11, or, in other words, it has prevented the obliteration of the angle in the body-wall due to inrolling, and has in fact made this angle less obtuse (cf. Figs. 12 and 13). If this conclusion be correct, and there is little room for doubt, it follows that the appearance of the supplementary disc is due, not to the presence of the cut, but to the bending inward of the body-wall in such a manner as to form in longitudinal section an angle opening aborally. But how can the formation of this angle determine this region for the formation of tentacles? The only answer to this question which seems to me at all satisfactory is the suggestion already made (Child, '04*b*, '04*c*, '04*d*) that the circulatory currents passing orally along the body-wall in each intermesenterial chamber strike against the inrolled body-wall and so produce areas of increased pressure to which the tissues react by growth and tentacles appear. The arrows in Figs. 11–14 indicate these currents. In no case within my experience have tentacles been seen to arise otherwise than on an inrolled portion of the body-wall. Ordinarily of course the inrolled region is a cut edge, but here the cut closed before the inrolling was brought about to any great extent.

If the formation of the angle in the body-wall instead of the presence of a cut surface is the essential condition for tentacle formation, it should be possible to bring about tentacle formation at any level capable of growth by constricting the body just oral to this level. I have attempted many times to carry out experiments of this kind by ligaturing the body at some level. All of these attempts have failed because the specimens parted in the plane of the ligature after a few hours if this was tied tightly, or crept out of it if it was at all loose. I am firmly convinced, however, that the production of these supplementary discs and tentacles at any level of the body capable of growth is possible without the presence of a cut surface, and I predict that as soon as a method is devised for retaining a sufficient local constriction at the same level for a few days without causing injury to the body-wall, the production of tentacles without relation to cut surfaces will be possible in *Cerianthus*.

Like the tentacles on the lower margin of an oblique piece (Child, '04*d*), the tentacles below a lateral cut when once growth has begun, continue to grow even though the animal is removed from the netting. By this time union of body-wall and mesenteries in the new relations is sufficiently advanced to cause the persistence for a time of the fold formed by the netting. In most cases, however, the fold is gradually obliterated by the internal pressure and the supplementary disc protrudes from the side of the body (Fig. 15).

Loeb called attention to the fact that only those supplementary discs which are situated near the oral end of the body possess mouths, the others consisting merely of a partial ring of tentacles arising from a small surface resembling part of a disc but completely closed. Comparison of Figs. 2, 3, 4, 6, 7, 8 with Fig. 15 will show the difference in character of the supplementary structures at different levels. It is evident at once that the presence of the œsophagus is responsible for the formation of the new mouth in supplementary discs near the oral end. Aboral to the œsophagus there is nothing with which the cut edges of the body-wall can unite to give rise to a new mouth. If a cut passing nearly through the body is made aboral to the œsophagus then the inrolled cut margins aboral to the cut unite with the cut ends of mesenteries over a large part of the circumference and are held in the inrolled position long enough for the formation of a new mouth. As a matter of fact, however, in such cases the part of the body oral to the cut, being united with the aboral portion only by a slender strip of the body-wall; separates after a few days and the regenerating disc is no longer supplementary but terminal on the aboral piece and regenerates in the typical manner. Owing to this separation I have not yet succeeded in bringing about the production of supplementary discs with mouths in the region aboral to the œsophageal region.

As regards the relation between rapidity and amount of growth and the level at which these supplementary discs are situated, my own observations agree with those of Loeb. He found that the rapidity and amount of growth decreased with increasing distance from the oral end and that the extreme aboral region was incapable of producing supplementary discs. These results also agree with the observations described in my second paper (Child, '03*b*) concerning the effect of position upon rapidity and amount of regeneration.

General Considerations.

The formation of supplementary discs constitutes another chapter of evidence on the rôle of internal water-pressure in regulative processes in *Cerianthus.* It is scarcely necessary, I think, to devote further space to the consideration of Loeb's view that tentacle-reduction is due to loss of intracellular turgor. It certainly does not account for the observed facts to which Loeb attempts to apply it. A knowledge of the structure of the animal would have made such conclusions impossible.

These experiments show most clearly that continuous or nearly continuous tension due to internal pressure is absolutely essential for the persistence of form and structure in *Cerianthus.* In its absence atrophy always occurs.

Doubtless various changes in the intracellular turgor occur during reduction and atrophy but these must be regarded a result rather than a cause, for so long as the tissue is subjected to the tension caused by internal water-pressure these changes in turgor do not occur.

The fact that the changes in *Cerianthus* which Loeb regarded as change in turgor are not such does not of course detract in any way from the value of the osmotic hypothesis in general. It is important, however, that the effects of distension of internal cavities with water or other fluids should be distinguished from the effects of intracellular phenomena. The case in hand is simply an incorrect application of the osmotic hypothesis.

On page 57 Loeb ('91) makes the following statement regarding the supplementary disc formed in the middle region of the body: "Diese neugebildete Kopf, b. Fig. 2, scheint ganz auf dem Ektoderm zu sitzen." It would not have required an extended examination to show the incorrectness of this conclusion. These lateral discs are no more wholly ectodermal than the terminal discs of normal animals (see the figures of this paper).

Heteromorphic Tentacles.

Experiments.

In two cases among the hundreds of pieces examined the appearance of tentacles on the aboral end of a piece has been observed. Both cases occurred in a single series and were apparently due to closure of the pieces in a peculiar manner. Although numerous attempts to obtain additional cases were made, none were successful, the pieces failing to close in the proper manner. I have little doubt that whenever closure takes place in a certain manner to be described heteromorphic tentacles will be formed.

The pieces which afforded this peculiar result belonged to a series intended for the study of the possible methods of closure. The pieces were prepared as follows. A piece was cut oral to the middle of a specimen by two transverse cuts as indicated in Fig. 16. The oral end of the piece was in all cases just below the disc in the œsophageal region, the aboral end a considerable distance aboral to the aboral end of the œsophagus. The cylindrical piece thus obtained was divided longitudinally into halves as indicated by the small diagram to the right of Fig. 16. Each piece then represented half of the circumference of the body and œsophagus. The body-wall of the piece was bounded on all sides by cut surfaces, two transverse at the ends and two longitudinal at the sides. The portion of the œsophagus in the piece was bounded orally and laterally by cut surfaces, but aborally it terminated in the normal manner.

Pieces of this kind close in various ways. Some roll up longitudinally and form almost perfectly typical animals, others roll in part longitudinally and in part transversely and the cut surfaces of œsophagus and body-wall unite according to chance, *i. e.*, union of the adjacent cut surfaces occurs, whatever these may be. Many bizarre forms are produced, but they afford nothing new in principle. One important fact is shown by many pieces including the two which possess special interest at present, viz., that the aboral end of the œsophagus, although not a cut surface is capable of union with a cut surface. It is of interest in this connection to note that nowhere else in the body does the body-wall terminate in a free margin. This free margin behaves like a cut surface and may like any cut surface give rise to new tissue after union with another cut surface (see Child, '04*a*).

In cases where these pieces succeed in rolling up longitudinally so that the longitudinal cut surfaces of body-wall and those of the œsophagus unite, a more or less typical mouth and œsophagus result from the union and regeneration may proceed in an almost typical manner. Usually, however, the œsophagus unites with the body-wall as in œsophageal pieces (Child, '04*d*), either on all sides or partially. Aboral union between œsophagus and body-wall is made possible by the bending orally of the aboral part of the body-wall so that aboral end of the œsophagus and aboral cut surface of the body-wall unite. In all cases where union between œsophagus and body-wall occurs elsewhere than at the oral end a closed piece without a mouth is the result and a mouth cannot regenerate. Since there is no communication between enteron and exterior in such pieces it might be expected that they would behave as regards tentacle-regeneration like the œsophageal pieces (Child, '04*d*) *i. e.*, that they would become slightly distended at first and regenerate small tentacle-buds but would afterwards collapse and the tentacles would be reduced. As a matter of fact, however, these pieces though never approaching pieces which possess mouths often show a far greater power of tentacle-regeneration than œsophageal pieces. They frequently regenerate tentacles 5–6 mm. in length, *i. e.*, twice as long as those of the best œsophageal pieces. They may remain distended for a month or more but sooner or later collapse occurs and the tentacles undergo reduction. Since these pieces are completely closed like the œsophageal pieces they can become distended only as fluid passes through the body-wall ('04*d*). The important point is that the distended condition continues for a much longer time than in œsophageal pieces. The reason for this difference is probably to be found in the fact that pieces cut as in Fig. 16 contain not only the entoderm of the œsophageal region but the great mass of mesenterial differentiations aboral to the œsophagus, *i. e.*, the chief digestive region of the entoderm. There is little doubt that processes of secretion are much more active and persistent in this region than in the œsophageal entoderm, and consequently the quantity of fluid reaching the enteron either in connection with the secretory process or by osmosis through the body-wall in consequence of the presence of soluble substances secreted into the enteron is greater than in œsophageal pieces and distension continues for a longer time. Whatever the exact explanation may be the fact is patent in all cases. As will appear below, it is perhaps of considerable importance in connection with the appearance of heteromorphosis.

The pieces of this series were prepared September 26, 1902. All pieces were observed at intervals after the operation and among the methods of closure were found those represented diagrammatically in longitudinal section in Figs. 19 and 26. The body-wall and the œsophagus are indicated as in previous figures. In Fig. 26 the aboral end of the body-wall has bent over orally much farther than in Fig. 19. In both cases, however, the margin of the body-wall has united on all sides with that of the œsophagus, thus forming a closed piece composed partly of œsophagus and partly of body-wall. The enteric cavity of this piece is of course the enteric cavity of the lateral half of the body in the region from which it was taken. In Figs. 19 and 26 the external surface of the œsophageal portion on the right of the figure is ectodermal, but in the normal animal formed the axial surface of the œsophagus. The only essential difference between such pieces and œsophageal pieces (Child, '04*d*) consists in the greater length of the body-wall in the former.

Tentacle-regeneration at the oral ends of the pieces occurred in the typical manner (Figs. 20 and 27), each producing about half the number of tentacles possessed by the parent specimen, a result to be expected from the number of intermesenterial chambers contained in each piece.

About two months later (November 20) two of the pieces showed tentacles beginning to develop on the aboral ends. One of these pieces is shown in Fig. 17. The tentacles on the oral end had already attained their maximum development and were undergoing reduction, and in the case of the marginal tentacles, atrophy. On the aboral end of the piece four tentacle-buds appear. These seem to arise either from the œsophageal tissue or from the line of union between the aboral end of the œsophagus and the body-wall. The relations of parts is illustrated by the diagrammatic longitudinal sections, Figs. 19–22. Figs. 19–21 show the closure and the course of regeneration during the first two months. Fig. 22 represents a section of the stage shown in Fig. 19, and here the relation of the aboral tentacles and œsophagus and body-wall is seen. During the following months

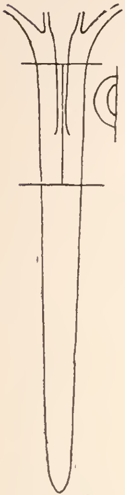
FIG. 16.

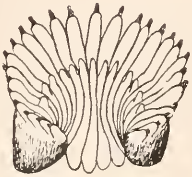
FIG. 17.

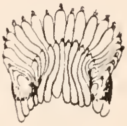
FIG. 18.

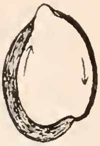
FIG. 19.

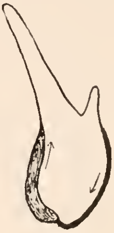
FIG. 20.

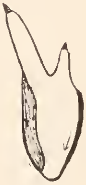
FIG. 21.

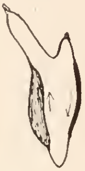
FIG. 22.

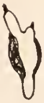
FIG. 23.

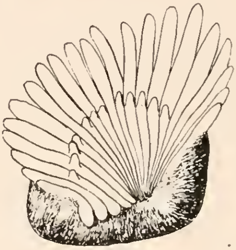
FIG. 24.

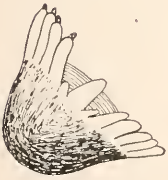
FIG. 25.

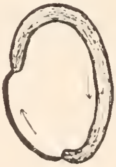
FIG. 26.

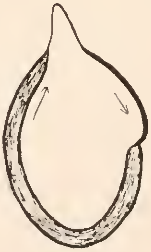
FIG. 27.

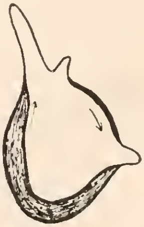
FIG. 28.

the number of aboral tentacles increased (Fig. 18, December 28) though the piece as a whole was undergoing reduction in size. Fig. 23 represents the section at this stage.

The other piece in which aboral tentacles developed is shown in Fig. 24 as it appeared two months after the operation (November 20). The method of closure in this piece differs somewhat from that occurring in the first: Figs. 26–28 illustrate this point. It will be seen that the aboral end of the body-wall bent orally much further than in the first piece (cf. Figs. 19 and 26), but also united with the aboral end of the œsophagus. It is probable that the piece was longer than the preceding. The difference in method of closure is readily accounted for by such difference in length. At the stage shown in Fig. 24 the aboral tentacles were beginning to develop. Fig. 28 is a diagrammatic section of this stage. Figure 25 represents a side view of the piece a month later (December 28). As the figure shows, the large tentacle in the middle arose just over the region where the body-wall folded longitudinally upon itself. (The shading of the body-wall represents the longitudinal striping.) All tentacles to the left of this large tentacle are upon the oral end of the piece, and all to the right are aboral. Six aboral tentacles appeared on this piece in addition to the single tentacle which can scarcely be regarded as either oral or aboral. In this case, as in the preceding, the aboral tentacles arose either from the œsophagus or from the line of union between it and the body-wall (Fig. 28).

Figs. 19–23 and 26–28 show that reduction of the body-wall is more rapid than reduction of the œsophageal region. This difference is probably correlated with functional differences in the tissues. Normally the body-wall is subjected to greater tension than the œsophagus, for the two sides of the latter are simply appressed during distension of the body. This being the case, it follows that conditions of tension depart less widely from the norm for the œsophagus than for the body-wall in these pieces, hence the more rapid change in the latter.

General Considerations.

These aboral outgrowths have been called tentacles for, in my opinion no ground exists for believing that they are anything else. It is evident from Figs. 17 and 24 that they arise in connection with intermesenterial chambers, for the lines extending aborally from the bases of the oral tentacles represent grooves marking the lines of attachment of the mesenteries to the œsophagus. Moreover, Fig. 24 shows that the aboral tentacles arise from those intermesenterial chambers which are least contracted for the only intermesenterial chambers which are at all distended at the aboral end are those from which the aboral outgrowths arise. In these respects these structures resemble the normal tentacles, and these facts lend color to the view that the conditions of origin of these aboral tentacles are similar to those of the normal tentacles.

In Figs. 19–23 and 26–28 the course of the intermesenterial circulatory currents is represented by arrows. These pass orally along the body-wall and aborally along the œsophagus. In both pieces the aboral end of the œsophagus bulges outward just oral to the line of union with the body-wall.

From Figs. 20 and 27 it is seen that this condition presents an obstruction in the course of the current in the aboral direction along the œsophagus, *i. e.*, local pressure upon the wall may occur. Except that they are reversed in position conditions here do not differ essentially from those described above leading to the formation of supplementary discs in the middle region (see Figs. 12–14). In brief, the suggestions regarding local pressure due to circulatory currents are as readily applicable to these cases as to the normal oral tentacles. Moreover, there is no adequate explanation on any other basis for the fact that these aboral tentacles arise from œsophageal tissue, or from the line of union between œsophagus and body-wall, rather than from the body-wall, like tentacles at the oral end. In my opinion the occurrence of these heteromorphic tentacles constitutes another important piece of evidence in support of the views already set forth (Child, '04*b*, '04*c*). The case of the single large tentacle which arose from the new tissue at the union of œsophagus and the folded body-wall (Fig. 25) is peculiar. The size of the tentacle is probably due to the greater distance between mesenteries, itself the result of stretching or growth of the new tissue at this point. The impossibility of ascertaining the exact internal condition in this case is evident. Opening of the piece must of course lead to extensive contraction, and fixation of these forms likewise causes extreme distortion as regards details, even after anesthesia. In external examination of the piece it could be seen, however, that a wide space between mesenteries existed here in the new tissue and from this region the large tentacle arose. What the course of the circulatory currents may have been it is also impossible to say. The parts which fused about this region were a part of the aboral end and a part of the longitudinal cut surface of the œsophagus, a part of the aboral end, the longitudinal cut surface and a part of the oral end of the body-wall. It is not impossible that both orally directed currents along the body-wall and aborally directed currents along the œsophagus may have been concerned. Moreover, regulative changes may have occurred in the distribution and direction of movement of the cilia. Thus, except for the great space between mesenteries, there is no definite evidence regarding the special factors concerned in the origin of this peculiar tentacle. Nevertheless, I think the conclusion is justifiable that the conditions of origin are not different in this case from those in cases already discussed. It is certain at any rate that some degree of distension is necessary for the formation of these aboral tentacles.

One important difference between the aboral tentacles and oral tentacles appears in both pieces, viz., the difference in time of appearance. In both cases the marginal tentacles at the oral end appeared within a few days after section, and the labial tentacles somewhat later, but the tentacles on the aboral end appeared only after about two months when the internal pressure was decreasing and the pieces were becoming reduced in size. This difference in the time of appearance directs attention to an important problem, viz., that of polarity so-called. Is the later appearance of the tentacles at the aboral end to be accounted for by a difference in physiological condition between the two ends such that under stimuli the oral end gives rise to tentacles in a much shorter time than the aboral end or is it possible that in consequence of the chance relations between mesenteries œsophagus and body-wall in the two cases cited the stimulus became effective only at a much later stage than at the oral end ?

The data afforded by the two pieces do not permit a decision between these alternatives. It is probable that extensive modifications of the greatly thickened and differentiated mesenterial margins aboral to the œsophagus occurred before relations between the intermesenterial chambers and the aboral end of the piece approached those at the oral end. If this is the reason for the late appearance of the aboral tentacles it is unnecessary to assume that the tissues of the aboral end are less capable of forming tentacles under proper conditions than those of the oral end or in other words the difference in the time of appearance of oral and aboral tentacles does not indicate the existence of a physiological polarity except so far as the structure at the aboral end was originally different from that at the oral end.

On the other hand it may be that the aboral ends of such pieces are inherently less susceptible to tentacle-forming stimuli and that, therefore, action of the stimuli during a much longer time than at the oral end is necessary. If it shall be possible in the future to obtain sufficient material of this kind for a thorough histological examination of the alterations in the mesenteries at the aboral ends of pieces we shall be better able, perhaps, to decide the question.

The œsophageal pieces described in a previous paper (Child, '04*d*) present conditions somewhat similar to those in the two pieces with heteromorphic tentacles, in that the œsophagus unites orally and aborally with the body-wall forming a series of closed intermesenterial chambers within which the circulatory currents pass orally on the body-wall and aborally on the œsophagus. If these currents are factors in tentacle-formation and if they are equal in force in both directions it would seem that tentacles must appear on both oral and aboral ends of such pieces at the same time. Yet in no case has such a result been attained. The œsophageal pieces regenerate short tentacles on the oral end, but the internal pressure soon disappears and regeneration is replaced by reduction. In the two pieces described in the present section the internal pressure continues for a much longer time than in the œsophageal pieces. It appears from this that œsophageal pieces might produce aboral tentacles if the internal pressure continued for a sufficiently long time. But even if this should prove to be the case the question as to polarity or structural conditions as the cause of the difference of time in the appearance of tentacles at the two ends would remain to be decided. In the œsophageal pieces, however, the structure and relations of mesenteries at the two ends are essentially the same, no more modification being necessary at one end than at the other to produce typical structural conditions for tentacle-formation. Since this is the case the absence of tentacles on the aboral ends of œsophageal pieces seems at present to indicate that the difference between the two ends as regards tentacle-formation consists in something else than gross structural relations of parts, *i. e.*, that some kind of polarity exists.

The nature of organic polarity is at present exceedingly obscure, but it is well known that the polarity of pieces as regards regeneration is dependent upon the previous relations of the pieces as parts of a whole. In pieces of the lower forms, such as *Hydra*, other hydroids, *Planaria*, etc., the polarity may not be manifest in any distinct structural differentiation at the two ends, but merely in their functional condition. The piece, retaining more or less completely the functional capacities of the whole from which it was taken, uses, or attempts to use, its parts in a similar manner, *i. e.*, the piece, in continuing its functional life, attempts in some degree to use its aboral or posterior end as the aboral or posterior end of the whole is used, and the same is true of its anterior end. The functional stimuli affecting these parts are similar to, though perhaps less powerful than those in the whole. It is probable that the regeneration of the characteristic structures at the two ends is closely correlated with these stimuli. When we can alter the conditions so that the aboral or posterior end is affected by external stimuli which typically affect the oral or anterior end while the "internal polarity" remains the same, the result depends upon the relative value of external and internal conditions as formative factors. Frequently in such cases the regeneration is delayed; apparently because of the "confusion" of stimuli, *i. e.*, the original stimuli and those resulting from the atypical orientation. Finally one or the other dominates and the "polarity" either remains as it was originally, or is reversed. In more highly organized forms, where internal factors are much more affective than external, reversal of polarity is rarely or never possible.

I think it probable that there is a difference in functional condition between the two ends of the pieces of *Cerianthus*, in consequence of which the one end is functionally oral and the other aboral. But if the aboral end be subjected to conditions which typically affect only the oral end, and if these conditions continue for a sufficiently long time, the response takes place and structures typically oral in character appear on the aboral end. Whether the inherent polarity or the altered external conditions will dominate in any particular case can be determined only by experiment.

SUMMARY.

1. Supplementary partial discs with a number of tentacles corresponding to that portion of the whole circumference which they represent can be produced from lateral transverse cuts in the body-wall of *Cerianthus*.

2. If the lateral cut is in the œsophageal region and is deep enough to involve the œsophagus the supplementary disc possesses a mouth because the cut surfaces of the body-wall unite with the cut surfaces of the œsophagus, thus forming a second opening into the œsophagus. If the cut is below the œsophageal region the supplementary disc possesses no mouth. In the extreme aboral region of the body the formation of supplementary discs does not occur.

3. When the lateral cut is made in the œsophageal region the permanent collapse and atrophy of the tentacles and region directly above the cut occurs, or if these have been removed their regeneration is retarded and atrophy occurs after a time. This collapse and atrophy is due, not to loss of intracellular turgor but to decrease in the internal water-pressure since the enteric cavity of the region above the cut is completely shut off from the general enteric cavity and from the exterior.

4. In two cases the formation of tentacles on the aboral end of a piece of certain form was observed. In both of these cases the conditions of internal pressure were apparently similar to those which exist at the oral end, but the less rapid regeneration of the aboral tentacles indicates either a difference in structural relations at the two ends or a difference in "polarity."

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December, 1903.

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BIOLOGICAL BULLETIN

COMPARATIVE PHYSIOLOGY OF THE INVERTEBRATE HEART.[1]

With Plates IV. to VIII.

A. J. CARLSON.

(From the Hull Physiological Laboratory, University of Chicago.)

I. The Innervation of the Heart.

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Before proceeding to describe the ganglia and the nerves that make connection with the cardiac apparatus particularly in the molluscs it seems necessary to make an explanation regarding the nomenclature to be followed. There is no little confusion in the terminology applied to the different ganglia and nerves connected with the viscera and the respiratory organs in the molluscs. This is, in a great measure, due to the difficulty in making out the homology between the ganglia and their connectives in the different groups, but also to the fact that names have been invented with sole reference to the *position* of the ganglia, not to mention the far-fetched homologies that have been made out between the molluscan and the vertebrate nervous systems. Thus Alder and Hancock (1842) apply the terms "cerebro-spinal" and "sympathetic" to the nervous system of the gasteropods. Chéron (1866) endeavors to make out an analogy between the stomato-gastric nervous system in the cephalopods and the sympathetic system in the vertebrates. And Ransom (1884) applies the term "vagi" to the two nerves from the pleuro-visceral ganglia to the cardiac and the branchial ganglia of *Octopus*. The discrepancies in these homologies between the molluscan and the vertebrate nervous system are so considerable that the introduction of the terms "cerebro-spinal," "sympathetic," "vagus," etc., serves rather to confuse. Scarcely more commendable is the introduction of names of ganglia with reference to their position only. Thus Bottazzi and Enriques (1901) reject the commonly accepted names "cerebral," "pleural" or "pallial" and "pedal" ganglia for the œsophageal nervous complex of *Aplysia*, and substitute the terms "dorsal," "lateral," and "ventral" ganglia respectively. These names fit the conditions in *Aplysia* admirably; but how will they apply to *Pleurobranchæa*, in which the pleural and the cerebral ganglia are fused into one ganglionic mass situated dorsally, or to the Dorididæ, in which both the cerebral, the pleural, and the pedal ganglia are fused into one ganglionic mass situated dorsally with reference to the œsophagus? It is plain that if we were to follow the principle suggested by Bottazzi and Enriques, different names would have to be invented to fit the conditions in the different subdivisions of the gastropods, and frequently even for different genera within the same group, because the position of some of these ganglia is subject to considerable variation. This objection applies also to the term "subanal," which is used by Lacaze-Duthiers (1859) for the ganglion commonly known as the visceral ganglion in the Haliotidæ.

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[1] Part of this work was carried out at the Physiological Laboratory of Stanford Universtiy, at the Hopkins Seaside Laboratory, Pacific Grove, Cal., at the Marine Biological Laboratory of the University of California at San Diego, and at the Marine Biological Laboratory, Woods Holl, Mass.; and I am indebted to the directors of these several laboratories — Professors Jenkins, Gilbert, Ritter, Whitman, and Lillie — for the privileges enjoyed. During the greater part of the time devoted to the work I held one of the Research Assistantships of the Carnegie Institution of Washington, D. C. The Carnegie Institution also placed one of the research rooms at the Woods Holl Laboratory at my disposal during the summer of 1904.

Bottazzi and Enriques reject the names, cerebral, pleural or pallial and pedal on the ground that they are physiological misnomers, and such they are to some extent. The same objection may be raised to the names "visceral ganglia" and "visceral nerves" or "pleuro-visceral commissures," because these ganglia innervate other structures besides the viscera and because some of the visceral organs are innervated by nerves from other ganglia. The stomato-gastric nervous complex of the cephalopods and the gasteropods is as much "visceral" as the two pleuro-visceral connectives with their peripheral complex of ganglia. This is recognized by Haller (1882), who calls the buccal ganglia of the prosobranchs the "anterior visceral ganglia." But this requires new names for the visceral nerves in the cephalopods and for the pleuro-visceral connectives in the gasteropods. The principle of naming the different ganglia and nerves with reference to the organs innervated by them is the correct one, as it will insure the greatest possible uniformity of names in the different groups. But our knowledge of the molluscan nervous system does not yet allow such a revision of the terminology. And for that reason I will make use of the names visceral nerves and visceral ganglia, inadequate and misleading as they are, rather than burden the literature with additional terms, which in all likelihood would prove to be but makeshifts.

In the dissections no attempts have been made to work out the innervation of other organs besides the heart, except in so far as the nerves to the cardiac apparatus are involved. In the figures the nerves to the various visceral and pallial organs are indicated for the purpose of orientation. The results of the dissections by aid of a good hand lens have not been supplemented by histological methods. Frequently nerves could be followed to the base of the auricles or on to the aorta but not on to the auricles and the ventricles themselves, although stimulation of the nerves showed that the nerves entered these organs. I have no doubt that in such cases the nerves could have been traced on to the cardiac musculature by aid of histological methods.

1. *The Lamellibranchs.* — Nerves to the cardiac apparatus of the bivalve molluscs have been described by Quatrefages (1849) for *Terredo*, and by Hancock and Embleton (1852) for *Mya truncata*.

Quatrefages describes two pairs of exceedingly small ganglia situated in the posterior part of the pericardial cavity. Nerves from these ganglia reach the base of the auricles and enter the musculature of these organs. Other branches from the ganglia ramify in the pericardium. A small nerve connects each pair of ganglia with the large pleuro-visceral ganglion on the ventral surface of the posterior adductor muscle. Hancock and Embleton describe "two small elliptical ganglia attached to the anterior and under part of the branchial (visceral) ganglia and united by commissures. These send filaments to the ovary and to the ventricle of the heart." The ganglia and the nerves are not figured, nor is it stated in what way the nerves reach the heart. List (1902) in his recent monograph of the *Mytilidæ* of the Gulf of Naples makes no mention of the innervation of the cardiac apparatus. He describes nerves to the renal organs and the reproductive glands, but follows them only a very short distance from the pleuro-visceral ganglia. Dogiel (1877) has described nerve cells in the auricles and at the auriculo-ventricular junctions of *Pecten* and *Anadonta*, but he does not make out their connection with any nerves and ganglia outside the heart.

My own work was done on *Mytilus californianus*, *Mya arenaria*, *Tapes staminea* and *Platydon cancellatus*. In *Mytilus* (Pl. IV., Fig. 1) I found a series of small ganglia that probably correspond to the cardiac ganglia described by Quatrefages in *Terredo*. A small nerve is given off from the dorsal side of each visceral ganglion (*vg*). These nerves (4) run dorsally for a short distance close to the anterior surface of the posterior adductor muscle and then turn anteriorly, reaching the dorsal body wall through a portion of the reproductive gland. At this point two slender branches are given off; the lateral branch (5) can be followed anteriorly in the body wall into the base of auricle, but it cannot with certainty be traced on to the auricular musculature. The other nerve (6) can be followed anteriorly on to the pericardium near the median line. The main branch of the nerve takes a median course in the body wall towards the median line, but before reaching the median line it bifurcates, one branch passing posteriorly (7) and one anteriorly in the dorsal body wall. At the point of bifurcation there is a small ganglion (8, 9). The branch that takes the anterior course enters a small ganglion situated dorsal to the hind gut at the junction of the body wall with the pericardium (10). The two ganglia are connected by a tiny nerve. Several small nerves are given off anteriorly from the ganglia to the pericardum and the gut, but I was unable to trace any of these branches on to the musculature of the ventricle. There are individual variations as regards the place of branching of the nerves and the two sides are usually not symmetrical. If the ganglia on the course of these nerves correspond to the "cardiac ganglia" of Quatrefages, there is this difference to be noted that in *Mytilus* the ganglia are not situated in the pericardial cavity and that branches of the nerves can be traced to the base of the auricles but not on to the ventricle. The two nerves in *Mytilus* are probably more of dorsal mantle nerves than of cardiac nerves, for even where the nerves ramify in the pericardium, the pericardium and the body wall are fused into one. In most of the gasteropods the renal and the cardiac nerves are united in one common trunk. In *Mytilus* the renal nerves are given off from the cerebro-visceral commissures (11) and they can be followed throughout the whole length of the kidney at the base of the gill, but I was unable to trace any of the branches on to the auricles or the ventricle.

The visceral nervous complex of *Mya* (Pl. IV., Figs. 2 and 3) differs somewhat from that of *Mytilus*. The visceral ganglia are fused into one with enlargements at the point of origin of the branchial nerves, and there are in most cases slight ganglionic enlargements at the cerebro-visceral commissures a little anterior to their union with the visceral ganglion. One or two small nerves pass laterally from each commissure to the ganglionic protuberance at the point of origin of the branchial nerves (Fig. 2, 3). From the commissures close to the visceral ganglion two small nerves take their origin (Fig. 2, 4). After an anastomosing with the nerves from the commissures to the sides of the visceral ganglion these nerves take a postero-lateral course on the surface of the kidney between the commissures and the branchial nerves. Branches of these nerves can be traced into the substance of the kidney and to the base of the auricle. Posterior to the point of origin of the branchial nerves another pair of tiny nerves are given off to the lateral surface of the kidney (Fig. 2, 6). From the dorsal side of the visceral ganglion two nerves are given off similar to those described in *Mytilus*. One of these nerves passes to the posterior adductor muscle and the dorsal body wall of the anal region; the other nerve (Fig. 2, 3) also sends branches to the adductor muscle but the main branch passes dorsally to the body wall where it takes an anterior direction and can be followed into the dorsal wall of the pericardial cavity. On its course branches are given off to the kidney, the aorta, and the rectum (Fig. 3, 3 and 4). This nerve is evidently homologous with the dorsal mantle nerve in *Mytilus* (Fig. 1, 4).

In *Tapes staminea* the visceral ganglion is situated in the angle made by the posterior adductor muscle and the adductor muscles of the foot. The ganglion gives rise to five pairs of nerves, viz., the posterior mantle nerves (1), the nerves to the siphon (2), the great osphradio-branchial nerves (4), the cerebro-visceral commissures (*cvcom*), and a pair of tiny nerves to the posterior adductor muscle (3). In the figure these last nerves are turned to the side so as to be represented in the same plane, as they turn in a dorsal direction almost at right angles to the other nerves. The commissures and the branchial nerves run parallel and close together for a distance of about one centimeter from the ganglion, and then the branchial nerves turn laterally and anteriorly into the osphradium and the gills. At the level of the pericardial cavity the commissures penetrate the reproductive gland, to which they send fibers. Close to the visceral ganglion each commissure gives off a small nerve (5) which runs parallel with it for a short distance and then turns laterally to enter the kidney and the heart. These nerves may be designated the reno-cardiac nerves, and are evidently homologous with the nerves marked 4 in Fig. 2. The renal organs occupy the space between the adductor muscles of the foot and the pericardial cavity and extend along the base of the gills. The nerves branch so extensively in the kidney that I was not able to follow any one branch directly on to the auricles, but the physiological evidence is conclusive that these nerves send branches to the heart. The nerves to the ventricle enter that organ solely through the auricular walls, as severence of the anterior and the posterior aortæ together with the rectum at either end of the ventricle does not interfere with the influence of the nerves on the heart, but after the auricles have been severed, leaving the other connections intact, stimulation of these nerves or the visceral ganglion has no further effect on the heart.

Platydon differs from *Tapes* in that the reno-cardiac nerves (3) are given off from the visceral ganglion instead of from the commissures. A small ganglion (4) can also be made out on these nerves at the base of either auricle.

In *Venus*, *Cardium*, *Hennites* and *Pecten* the cardiac nerves were not worked out in detail, but the heart of these lamellibranchs is innervated from the visceral ganglion or the cerebro-visceral commissures just as in *Mya* and *Tapes*, as will be shown by physiological experiments in a subsequent paper. This is evidently the plan of the reno-cardiac innervation in all lamellibranchs. The cell bodies of the cardiac nerves are probably situated in the visceral ganglion or ganglia, the giving off of the nerves from the cerebro-visceral commissures instead of from the ganglion directly is evidently only a case of the fibers following the course of the commissures for some distance before turning laterally to enter the kidney and the heart. While it is certain that the heart of these molluscs is innervated from the visceral ganglion, the nerves entering the heart at the base of the auricles, the exact course of these nerves through the renal organs as well as in the heart itself remains to be worked out. This must be left to biologists who have more suitable material, like Tridacna, at their disposal.

Young (1881) studied the effect on the heart of the stimulation of the visceral ganglion in the lamellibranchs *Mya*, *Anadonta* and *Solen*. He gives no definite description of the course of the nerves from the visceral ganglion to the heart. He mentions two tiny nerves which he could trace from the ganglion on to the pericardium and the rectum, thinking that these were the cardiac nerves. From his statement that branches from these nerves pass to the rectum he evidently refers to nerves similar to those indicated by me in *Mya* and *Mytilus* as dorsal mantle nerves (Fig. *1*, 6, 10; Fig. *3*, *2*); but the physiological evidence goes to show that these nerves do not enter the heart.

2. *The Chitons.* — The chitons present a more diffused nervous

system than any other gasteropod. There are no definite ganglia, the nerve cells being scattered all along the main nerves or "nerve-cords." So far as I am aware, nerves to be cardiac apparatus of these animals have not been described. Haller (1883) describes nerve cells and nerve fibers in the walls of the auricles, but he does not make out the connection of these nerve fibers with any nerve or nerve-cord outside the heart. My own work was done on *Cryptochiton stelleri.* This species was selected because of its large size, individuals being frequently found that measured 30 cm. in length. From the lateral and ventral side of the œsophageal nerve ring (Pl. IV., Fig. 6) proceed two pairs of nerve-cords. The median pair passes posteriorly into the foot, the lateral pair runs posteriorly in the mantle in a sinus between and slightly dorsal to the efferent and afferent gill sinuses, to unite at the posterior end of the mantle. These are the pleuro-visceral cords. From these cords numereus nerves are given off, some taking a lateral and ventral direction to the gills, others passing dorsally into the mantle and median into the wall of the body cavity. The nerves vary in size, but they are all very small and branch soon after leaving the cord, so that it is difficult to follow them for any great length. In the region of the heart one and sometimes two of these tiny nerves can be followed in the wall of the efferent gill sinuses and on to the auricles (Fig. *6*, *1*). On their course these nerves branch like the rest so that only a small division of the nerve reaches the auricle and this branch cannot be followed in the auricular musculature for any considerable distance. These are, however, probably not the only nerves that reach the auricles from the pleuro-visceral cords. The auricles are closely attached to the body wall along the side or base, Haller (1883) even claiming that the auricular musculature shades imperceptibly into that of the body wall. Posterior to the opening of the efferent gill sinuses into the auricles, several nerves can be followed to within less than 1 mm. of the place of their attachment to the body wall (Fig. *6*, *2*). It seems therefore probable that the auricles are supplied, not with one but with several nerve filaments from the nerve cords. No nerves could be traced to the heart or the pericardium from the pedal cords.

3. *The Diatocardic Prosobranchs.* — Nerves to the heart of the diatocardic prosobranchs have been described by Lacaze-Duthiers (1859), Haller (1883), Bouvier (1889), and Illingworth (1902). Lacaze-Duthiers describes in *Haliotis* four nerves from the pleuro-visceral connectives and the visceral ganglion to the pericardium. He calls these cardiac nerves, but he does not trace them to the auricles or the ventricle. Haller finds that the heart of *Fissurella*, *Turbo* and *Trochus* is innervated from two different ganglia. He traces nerves from the branchial ganglia along the efferent gill sinuses to the auricles, while the ventricle is innervated by a nerve from the visceral ganglion that reaches the ventricle along the aorta. Bouvier describes similar nerves to the auricles and the ventricle of *Patella*, *Nerita* and *Helicina*, but he does not figure the course of the nerve from the visceral ganglion to the ventricle nor does he state whether the nerve enters the ventricle along the rectum or at the aortic end. Illingworth states that the cardiac nerves described by Haller and Bouvier are also present in *Lucapina*.

My own work was done on *Lucapina crenulata* and *Haliotis cracherodii*. In *Haliotis* (Pl. V., Fig. 7) two nerves (7 and 8) can be followed from the visceral ganglion or from the pleuro-visceral commissures close to the ganglion, to the renal organ and the base of the auricles. The nerves were not traced on to the auricular musculature, but I have physiological evidence that motor nerves from the visceral ganglion supply the auricles and it is probable that they reach the auricles in the trunk of the nerves 7 and 8. The visceral ganglion also gives off two nerves to the pericardium, one (9) passing to the dorsal, the other (10) in the ventral wall. The latter nerve sends one or two branches on to the aorta, but I did not succeed in tracing these branches on to the ventricular musculature. It can be shown physiologically, however, that motor nerves from the visceral ganglion enter the ventricle at the aortic end, and it is probable that these nerve fibers reach the organ in the ventral pericardial nerve. The innervation of the ventricle of *Haliotis* is consequently the same as that described by Haller for *Fissurella*, *Turbo* and *Trochus*, a nerve from the visceral ganglion entering the ventricle at the aortic end. I did not succeed in tracing any nerves from the branchial ganglia to the auricles, nor did I find any physiological evidence of their existence. If such a nervous connection exists, the nerves are evidently only sensory in function. The auricles are, however, innervated from the visceral ganglion.

The nervous system of *Lucapina* differs from that of *Haliotis* chiefly in the presence of two large ganglia, the intestinal ganglia, on the course of the pleuro-visceral commissures. From the posterior side of the visceral ganglion (Pl. V., Fig. *8*) a comparatively large nerve passes in a dorsal direction in the wall of the basibranchial sinus towards the rectum, but before reaching the rectum the nerve bifurcates, one branch passing on either side of the rectum to take an anterior course in the walls of the afferent gill sinuses. From the ganglion and from the nerve between the ganglion and the point of bifurcation two or three small nerves are given off (3, 4), and branches from these nerves can be followed into the pericardium. Illingworth states that he could trace some of these branches into the musculature of the ventricle along the rectum. I was not able to do so, and the physiological evidence is to the effect that the nerve enters the ventricle at the aortic end and not along the rectum. After the bifurcation, each nerve gives off a small branch that can be followed to the base of the auricle (5). From the gill ganglia several nerves are given off, but the only one that concerns our present inquiry is the tiny nerve described by Illingworth as passing posteriorly in the walls of the efferent branchial sinus, branching and anastomosing on its course, to finally end in the musculature of the auricle. I was able to follow the nerves (9) for some distance towards the base of the gills, but not on to the auricles. Stimulation of the efferent gill sinus does not influence the auricles, so that if this nervous connection, described by several investigators for so many species, is present in *Lucapina* the nerves are in all probability only sensory in function. The auricles of *Lucapina*, like those of *Haliotis*, are innervated from the intestino-visceral commissures or the visceral ganglion, as shown by stimulation of the commissures. The exact course of these auricular nerves remain to be worked out.

4. *The Monotocardic Prosobranchs.* — Nerves to the heart of the monotocardic prosobranchs have been described by Haller

(1882) and Bouvier (1889). Haller worked on *Murex trunculus.* In this mollusc there are three visceral ganglia. From the middle and largest ganglion a nerve passes along the aorta to the ventricle, while the auricle is innervated from the nerve plexus in the efferent gill sinus in the manner described by the same author for *Fissurella.* Bouvier worked on several genera but he describes the innervation of the heart particularly in *Cyclophorus tigrinus* and *Triton variegatum.* In *Cyclophorus* he describes a nerve to the ventricle only. "A gauche du ganglion (viscéral), la commissure viscérale donne naissance à un gros nerf cardiaque qui suit un instant l'aorte anterieure et penetre ensuite dans le ventricle. On le voit tres bien entrer dans l'organe sur la preparation que j'ai conservie, et je pense qu'il n'est pas un type plus commode pour l'étude de l'innervation du coeur" (p. 77). In *Triton* there are two visceral ganglia connected by a commissure. This commissure gives rise to three small nerves, one of which passes to the branchial vein, another to the auricle, and the third to the ventricle. This last nerve is given off by the commissure close to the right visceral ganglion. It runs for some distance in the pericardium and enters the ventricle at the aortic end.

My own work was done on *Natica lewissii* and *Sycotypus canaliculatus.* In *Natica* (Fig. 9) the right pleuro-visceral commissure is much stouter than the left. It enters the large branchial ganglion on the left side, which gives rise to four nerves, namely a comparatively large nerve to the osphradium and gill (3), a smaller one to the floor of the pallial cavity (2), a somewhat larger branch to the gill (4), and finally a small branch to the left visceral ganglion (5). On the course of this last nerve is a small ganglion (7) which sends a tiny nerve to the ventral wall of the pericardium (6). The two visceral ganglia are connected by a commissure. The right ganglion is much larger than the left. From the former two and sometimes three nerves take their origin, the largest of which divides immediately after leaving the ganglion, the main branch passing to the reproductive glands and the viscera (8), the smaller branch turning forward and laterally into the ventral wall of the pericardium (9). Branches from the two pericardial nerves (6 and 9) can be followed to the base of the auricle, to the junction of the aorta with the ventricle, and into the kidney. This arrangement is also born out by the physiological experiments. Each of the two nerves sends fibers to the auricle and to the ventricle by the aortic end. There is some individual variation as regards the point of origin of the genital and the reno-cardiac nerves from the right visceral ganglion. In some specimens the nerves do not leave the ganglion close together as indicated in Fig. 9, Pl. V., but the ganglion is distinctly bilobed and the genital nerve takes its origin from the anterior lobe near the entrance of the commissure. The innervation of the heart in *Natica* thus agrees in main with that of *Murex*, *Cyclophorus* and *Triton* as described by Haller and Bouvier. There is complete agreement in the innervation of the ventricle, nerves from the visceral ganglion or from the visceral commissure enter the ventricle at the aortic end. If the auricular nerve from the nerve plexus in the branchial sinus, as described by Haller in *Murex*, is present in *Natica*, it is only sensory in function, because stimulation of the branchia ganglion or the nerve in the efferent gill sinus (4) has no effect on the auricle.

The innervation of the heart of *Sycotypus* (Fig. 10) presents some differences from that of *Natia*, and *Sycotypus* being the larger animal the nerves and the ganglia can be worked out with greater accuracy. The right pleural ganglion gives rise to three nerves, two of which (*10*) unite to enter the osphradial and branchial ganglia. The third constitutes the left pleuro-visceral commissure. On the course of this commissure some distance from its entrance into the visceral ganglion in a slight ganglionic swelling giving rise to a tiny nerve which ramifies in the floor of the branchial chamber along the line of attachment of the gill. One of its branches turns posteriorly towards the base of the gill and the auricle, but I could not trace it on to the auricle itself. A similar tiny ganglion on the left commissure (*11*) gives rise to a nerve which enters the rectum and adjoining structures. The left visceral ganglion, the smallest of the two, gives rise to three or four small nerves, which ramify in the connective tissue making up the anterior and ventral wall of the pericardial cavity. One branch (*2*) can be traced to the base of the auricle, another branch (3) communicates with the ganglion on the ventriculo-aortic junction (8). The large visceral ganglion on the right side also gives rise to several nerves, one of which (5) can be followed with the unaided eye through its course in the pericardium till it enters the ganglion on the ventriculo-aortic junction. Branches from nerve 7 enter the renal organ. Nerve 4 enters the reproductive gland and the liver. Apart from nerve 5, which plainly enters the ventricular ganglion and is therefore, a cardiac nerve, there are several branches from nerves 4 and 6 that ramify in the pericardium. Whether any of these branches enter the auricle at its base I am unable to make out.

The ganglion on the ventriculo-aortic junction is large enough to be seen by the naked eye, and by the aid of a dissecting lens nerves can be traced from the ganglion for some distance on the ventricle (9).

There is no evidence of nerve-fibers passing from the branchial ganglion to the auricle. Either end of the heart is supplied with nerves from the visceral ganglia, and this is born out by the physiological experiments. The commissure between the two visceral ganglia is partly ganglionic and pigmented like the visceral ganglia themselves.

5. *The Tectibranchs.* — In his description of the nervous system of *Pleurobranchus* Lacaze-Duthiers (1857) does not come to any definite conclusion regarding the innervation of the heart. "Sur l'oreillette, on trouve des filets nerveux évidents, mais qu'il m'a été impossible, vu leur delicatesse, de les suire jusqu'à leur origin" (p. 285). But he believes that the auricular nerve makes connection with the right peripheral nerve from the visceral ganglion. This nerve is called by him the genital nerve. Dogiel (1877) describes in *Aplysia* a nerve to the auricle from the left and posterior side of the visceral ganglion. Vayssière (1879) has figured and described the nervous system of *Gasteropteron*, *Doridium*, *Philene*, *Scaphander* and *Bulla*, all of which belong to the family Bullididæ. Of the visceral organs he describes the innervation of the œsophagus, the stomach and the reproductive organs, but he makes no mention of the innervation of the heart. Ransom (1884) has shown that the left side of the visceral ganglion of *Aplysia* gives origin to two instead of one nerve as figured by Dogiel.

The anterior of these two nerves supplies the reproductive organs. The other nerve runs towards the gill, where it bifurcates, one branch entering the gill; the other "appears to end in the pericardium near the origin of the auricle." In his recent monograph of the Aplysidæ Mazzarelli (1893) describes and figures three nerves from the left side of the visceral (called by him deutovisceral) ganglion. Of these nerves the one given off furthest posterior "manda un sotil ramo alla membrana viscerale" (p. 108); but no reference is made to the innervation of the heart. Pelseneer (1893) has described the nervous system of *Bulla striata* and *Pleurobranchæa meckeli*. In *Bulla* he dot not figure the peripheral nerves from the two visceral ganglia (called by him supra-intestinal and abdominal ganglia), and consequently not the innervation of the viscera and the heart. In *Pleurobranchæa* he describes two nerves from the visceral ganglion. The one on the right side passes to the gill. The left nerve is called the genital nerve and the small ganglion on its course the genital ganglion, but the nerves peripheral to this ganglion are not described and consequently not the cardiac innervation. The most recent observations in this field have been make by Bottazzi and Enriques on *Aplysia* (1901). These authors describe a nerve from the right side of visceral ganglion to the aorta and from the left side of the ganglion "un nerf cardiaque, qui donne un rameau au nephredion et un qui va au second estomac triturateur (?)" (p. 122).

The presence of ganglion cells in the heart of *Aplysia* has been affirmed by Dogiel (1877) and denied by Ranson (1884).

My own work was done on *Aplysia californica*, *Bulla globosa* and *Pleurobranchæa californica*. In *Aplysia* (Pl. VI., Fig. 11) the pleural or pallial ganglia of the œsophageal complex give rise to two stout nerves, the pleuro-visceral commissures, which run free in the body cavity of the neck to enter into connection with the large visceral ganglion situated in the body cavity anterior to the visceral mass. From the right and posterior side of this ganglion proceeds a stout nerve (1) which enters the osphradium and the roof of the gill chamber. The left and anterior side of the ganglion gives rise to three nerves. The most anterior one (5) enters the mantel and the dorsum of the anal region. The middle nerve

(4) supplies the gill. These two nerves reach their destination by a circuitous route, passing ventral to the heart. The third nerve (3) is the visceral nerve par excellence, because it sends branches to the liver, the reproductive glands, the heart and the renal organ. Arising from the postero-lateral side of the ganglion this nerve takes a direction towards the aortic sinus and the pericardium, but before it reaches these structures it bifurcates. The branch that passes ventral to the aortic sinus (7) gives off a branch to the liver (10) and one or two very small branches to the aortic sinus (12), while the main trunk (11) follows an artery to the reproductive glands. The other nerve (6) takes a postero-lateral direction in the ventral wall of the pericardial cavity. On reaching the postero-lateral side of the pericardial cavity it takes a median direction parallel with long axis of the heart. Several small branches are given off by the nerve to the pericardium, and at the level of the auricle a tiny branch (9) is sent forward in the ventral wall of the pericardium and on to the auricle, while the main branch (8) enters the kidney. The nerve on the aortic sinus (8) could not be traced on to the ventricular musculature with certainty, but we shall presently see that nerves do enter the ventricle at the aortic end and that these nerve fibers leave the visceral ganglion in the trunk of the posterior nerve of the left side of the ganglion (3). From the posterior side of the visceral ganglion one or two small nerves (14) are given off which ramify in the musculature and connective tissue of the dorsum immediately adjoining the ganglion and cannot be followed for any distance. From the right pedal ganglion arises a small nerve (13) which connects with the right side of the visceral ganglion. The corresponding nerve of the left side does not come into connection with the visceral ganglion but enters directly into the musculature of the dorsum a little anterior to the pericardial cavity.

Electrical stimulation of either of these two nerves produces none of the effects caused by the stimulation of the pleuro-visceral connectives.

The visceral nervous system of *Bulla* (Pl. VI., Fig. 13) differs from that of *Aplysia* mainly by the presence of five or six ganglia on the course of the nerves. The left commissure has a small ganglion on its course before reaching the main visceral ganglion.

At this point a small nerve (2) is given off to the dorsum of the neck. In some specimens the ganglion is not situated at the point of origin of the nerve but nearer the visceral ganglion. The right visceral ganglion gives off peripherally one stout nerve (5), which enters the osphradium; here it divides and sends branches into the gill and to the roof of the gill chamber. The left ganglion gives off a corresponding nerve (6), which also bifurcates soon after leaving the ganglion. At the point of bifurcation is a small ganglion. One of the branches enters the inferior pallium, the other the posterior dorsum. Either visceral ganglion gives rise to another nerve (3, 4), much smaller than the one that takes its origin at the posterior end. These nerves unite in a small ganglion (7) situated to the left of the two main ganglia. This ganglion is called the "genital ganglion" by Vayssière, which is not a well chosen name, because the nerve that leaves the ganglion supplies not only the reproductive glands but also the renal organ and the heart. The nerve (8) that is given off by the ganglion soon bifurcates and at the point of bifurcation is another ganglionic enlargement (9). The main branch (10) takes a lateral direction and enters the genital glands, the other passes towards the pericardial cavity. This branch of the nerve sends a small filament on to the aortic sinus (11), while the main trunk passes posteriorly and median in the pericardium ventral to the heart, finally entering the kidney (14). Several small branches are given off to the pericardium, one of which can be followed to within one or two mm. of the base of the auricle, but not on to the auricle itself. The branch to the aortic sinus ramifies on the sinus making almost a complete ring at the ventricular junction. A branch can be followed on to the ventricle. In *Aplysia* I was unable to trace a nerve on to the ventricle, in *Bulla* I failed to trace the nerve to the auricle; but the physiological evidence is conclusive that fibers from the nerve (8) enter the auricle of *Bulla* at its base. The connection is probably with one of the tiny branches in the ventral wall of the pericardium.

In *Pleurobranchæa* (Pl. IV., Fig. 12) the pleural ganglia constitute the postero-lateral portion of the supra-œsophageal ganglion. The first pair of nerves that proceeds from the posterior and lateral side of the ganglion enters the mantle, the nerve on the right side also sending branches to the gill. From the second pair of lateral ganglionic protuberances are given off three nerves, one from the right side and two from the left. The posterior one on the left side, the larger of the two, enters the posterior part of the mantle of the same side. The other nerve (*lvn*) follows the course of the cerebro-pedal and pedal commissures towards the right side, where it takes a posterior direction and joins the visceral ganglion situated anterior and to the right in the body cavity. The corresponding nerve on the right side (*rvn*) is much stouter and is probably homologous to the two nerves given off from the corresponding ganglionic protuberance on the left side. Peripherally the visceral ganglion gives off two nerves. The nerve on the right side (1), the largest of the two, passes posteriorly in the body cavity at the margin of the mantle and the foot and enters the gill. The left nerve passes dorsally towards the pericardial cavity, a little in front of which it enters a small ganglion (3), designated by Lacaze-Duthiers as the "genital ganglion," which is certainly a misnomer, because the ganglion supplies other organs besides the reproductive glands. Five nerves are given off by this small ganglion. One of these (10) passes on to the anterior aorta and follows it along its ventral side to the aortic sinus, where it bifurcates, one branch (12) passing along the lateral, the other (13) along the posterior aorta. In large specimens a small ganglionic swelling can be made out at the place of branching (11). A tiny branch (14) is given off by this ganglion to the aortic sinus, but I did not succeed in tracing it on to the ventricle. The smallest nerve of the five bifurcates soon after leaving the ganglion, sending one branch (9) on to the ventriculo-aortic junction, the other branch (8) is lost in the connective tissue surrounding the ganglion. The nerve given off from the median part of the ganglion (5) is larger. It passes posteriorly to the auriculo-ventricular junction where it penetrates the muscular bands which make up the ventral wall of the pericardium and enters the auricle. Just as it enters the auricle a branch is given off to the ventral wall of the pericardial cavity. The main part of the nerve can be followed in the wall of the ventricle almost around the entire organ. It breaks up in several branches

(7) which pass towards the base of the auricle and perhaps into the venous sinuses between the auricle and the gill. In the largest specimens one or two of these branches can be traced to the base of the gill. This nerve is probably homologous to the nerve in *Aplysia* and *Bulla* which runs in the ventral wall of the pericardial cavity and supplies the heart and the renal organ. Parallel to this nerve runs an extremely small branch (6), sometimes given off from the ganglion, sometimes from the auricular nerve itself. The branch enters the pericardium. And lastly, the ganglion gives off a comparatively large nerve which takes a posterior and median direction and enters the genital glands (4).

6. *The Nudibranchs.* — The innervation of the viscera in *Doris tuberculata* has been described in great detail by Hancock and Embleton (1852). Four visceral nerves take their origin from the right and ventral side of the supraœsophageal ganglionic mass. One of these nerves sends branches to the renal organ, the gill, the vesicle ventral to the pericardial cavity (believed by these investigators to be a "portal heart"), and to two ganglia situated "on the apex of the ventricle." Besides these two ganglia on the ventricle they describe a great number of small ganglia on the peripheral course of the visceral nerves. Pelseneer (1893) designates two small nerves from the abdominal ganglia in the two Dorididæ *Polycera* and *Goniodoris* as "reno-cardiac nerves," but he does not follow them to their entrance of these organs.

My own work done on the three Dorididæ, *Montereina nobilis*, *Triopha carpenteri* and *Triopha grandis*. *Montereina* is the largest nudibranch at my disposal, and for that reason best adapted for working out the cardic innervation and for isolating the cardiac nerves for the physiological experiments. In this mollusc (Pl. VI., Fig. 14) the œsophageal chain of ganglia as well as the greater part of the visceral ganglia are fused into one supraœsophageal ganglionic mass, the more primitive arrangement of the ganglia being indicated by the presence of the stout commissure which passes under the œsophagus and connects both sides of the ganglion. From the right and ventral side of this composite ganglion near the origin of the œsophageal commissure a nerve (*vn*) is given off, which takes a posterior direction ventral to the two posterior nerves to the mantle. On reaching a branch of the anterior aorta it follows the latter in a median direction towards the hind gut, where it bifurcates. The smaller branch (1) continues in the median direction along the artery and, passing ventral to the hind gut, it can be followed to the left ventral side of the aortic sinus. The main branch passes posteriorly on the left side of and close to the hind gut. At the level of the ventricle it gives off a small branch (2) which passes in a median direction dorsal to the hind gut and enters a small ganglion situated on the aortic sinus (6). From this ganglion one or two branches can be traced on to the aorta and another on to the ventricle. This ganglion evidently corresponds to the two ganglia described by Hancock and Embleton in *Doris tuberculata* as situated on the "apex of the ventricle." The main branch of the nerve continues posteriorly along the gut to the anal region. On this course the nerve gives off a branch (3) to the ventral wall of the pericardium and probably also to the renal organ. The terminal branches of the nerve (4, 5) pass to the base of the auricle and to the gill. There appears to be a small ganglionic swelling on the nerve before it breaks up into the gill branches. I was not able to follow the nerve into the auricle, but the physiological evidence show clearly that nerve fibers from this nerve enter the auricle at its base.

In *Triopha carpenteri* (Pl. VI., Fig. 15) the central nervous system is fused into one ganglionic mass situated dorsal to the œsophagus, just as in *Montercina*. From each postero-lateral horn of the ganglion proceeds one nerve which innervates the mantle (1, 2). From the posterior and right side of the ganglion, ventral to the origin of the right mantle nerve, four small nerves take their origin. The destination of two of these (5, 8) could not be made out with certainty, but the other two nerves supply the viscera. The nerve furthest to the right (7) enters a small ganglion (*gi.*) situated on the vagina. This ganglion sends branches to the copulatory organs and the reproductive glands. The other nerve (6) passes posteriorly in the median line dorsal to the visceral mass. On this course the nerve gives off several tiny branches which cannot be followed for any distance from the nerve on account of their minuteness. The largest branch probably enters the intestine and the liver (9). The main trunk of the nerve is continued posteriorly to within a short distance of the large forward loop of the intestine where it bifurcates, the smaller branch (10) passing along the anterior aorta ventral to the intestinal loop, the larger branch (11) turning slightly to the right reaches the base of the auricle and the gill along the rectum on the right side of the pericardial cavity. I could follow the smaller branch (9) along the aorta to within 2 mm. of the ventricle, but not on to the ventricle itself; nor could I trace any branch from the other division of the nerve on to the auricle. Electrical stimulation of the nerve 7 shows that fibers from this nerve reach both auricle and ventricle. The course of this nerve (7) is very similar to that of the "visceral" nerve in *Montereina* (Pl. VI., Fig. 14, *vn*). The left side of the supracœsophageal ganglion gives rise to a small nerve (4) which innervates the stomach, and probably also the digestive glands and the intestine.

Triopha grandis attains to a considerably greater size than either of the two foregoing Dorididæ, but it is much less abundant. I have obtained only a few specimens of this nudibranch at Monterey Bay during the months of July and August, when it comes into shallow water to breed. I was therefore unable to make any physiological experiments on the heart and the heart-nerves, but sufficient material was at my disposal to work out the cardiac nerves anatomically. This appears to be essentially the same as that of the previous species, only that the greater size of this mollusc enables one to follow the nerves to their terminations with greater accuracy. Nerves 1 and 2 (Fig. 16) take their origin on the posterior and lateral sides of the brain and pass laterally and posteriorly on the dorsal surface of the visceral mass close to the visceral envelop, on this course sending numerous branches to this envelop and to the dorsum. At the level of the sinous passages from the gill to the auricle the two nerves enter two small ganglia situated in the angle made by the base of the auricle and the rectum (9). These ganglia are joined by a commissure. From each ganglion a tiny nerve passes dorsally and anteriorly evidently entering the pericardium and the auricle. Stouter branches pass posteriorly into the gill. These branches have several small ganglia on their course.

Nerve 3 takes its origin slightly dorsal to the right branchiomantle nerve and takes a course lateral to it. On reaching the anterior or cephalic artery the nerve bifurcates, the smaller branch (4) continuing posteriorly to the genital organs. The final destination of this nerve was not determined, but it does not come into relation with the heart. The main branch follows the artery, ventral to it, in a median direction, and at the level of the forward loop of the intestine it bifurcates, the smaller branch passing dorsal to the gut, its branches ramifying in the visceral envelop and on the surface of the liver. None of its tiny rami can be traced to the region of the paricardium and the heart. At the place of bifurcation is usually a small ganglionic swelling (5). The main branch continues posteriorly along and ventral to the artery and can be followed on to the junction of the aortic sinus with the ventricle. Branches from this nerve pass to the main arteries and probably follow them peripherally just as in *Pleurobranchæa*. In large specimens the branch to the ventriculo-aortic junction (7) can be traced on to the ventricle. This is, therefore, one of the cardiac nerves. The heart is thus supplied with nerve-fibers from nerve 3 and in all probability also from nerve 2 (and 1 ?) just like the two foregoing Dorididæ, in both of which nerves enter the heart at the aortic as well as at the auricular ends.

7. *The Pulmonates.* — Ransom (1884) has described nerves to the auricle and the ventricle of *Helix:*

" From the median protuberance on the œsophageal ganglionic mass comes off almost from the middle line a couple of visceral nerves. The larger of the two, the left nerve, runs along the aorta towards the ovisperm duct, to which it gives off a branch. It then divides, and one part follows the aorta into the ventricle while the other goes to the kidney, where it ramifies and some branches are continued to the origin of the auricle " (p. 327).

Curiously enough, Ransom figures the ventricle as being situated anterior to the auricle. Plate (1898) describes four nerves from the visceral (pleural) ganglion in the Janellidæ. Of these nerves three enter the pallial complex and the fourth supplies the renal organ. " Er versorgt in erster Linie die Niere. Ob er, ausserdem, wie wahrscheinlich ist, den Herzbeutel und das Herz, villeicht auch die Athmrören innerviert, bleibt weitern Untersuchungen zu Feststellung vorbehalten " (p. 256).

My own work was done on the two slugs, *Limax maximus* and *Ariolimax columbianus* and on the snail *Helix* (*Arionta*). In *Limax* (Pl. VII., Fig. 17) the pedal and pallial ganglia are fused into one ganglionic mass situated ventral to the œsophagus and connected with the supraœsophageal ganglion by a commissure on either side of the œsophagus. This pallio-pedal ganglion is pierced by the cephalic artery; the portion of the ganglion dorsal to the artery is the pallial ganglion proper. This dorsal portion of the ganglion gives rise to four nerves. The two lateral ones (1, 2), which are the stoutest, pass to the pallial complex and the mantle. Besides the mantle nerve, the left side of the ganglion gives rise to two smaller nerves, one of which takes a posterior direction close to one of the adductor muscles and ramifies in the dorsum at the place of attachment of these muscles. The other nerve (*vn*) runs posteriorly a little to the right of the copulatory organ. On this course a branch is given off to the salivary gland. At the level of the bifurcation of the aorta the nerve enters a small triangular ganglion (4), situated in the connective tissue close to the reproductive gland. The ganglion gives rise to three nerves. The smallest one of these (5) passes to the reproductive gland. A larger branch (7) takes a posterior and median direction along the posterior artery to enter the liver and the intestine. The largest branch (6) takes a dorsal and anterior direction towards the kidney, but before reaching that organ it bifurcates, both filaments entering the ventral side of the kidney. The nerve that follows the posterior artery (7) gives off two very tiny branches to the aorta, but I was not able to follow them on to the ventricle. Nor was I able to trace the nerves that enter the kidney to the musculature of either the ventricle or the auricle, although a branch can be followed to within 2 mm. of the base of the auricle. The physiological experiments prove, however, that fibers from the visceral nerve enter the auricle and the ventricle at the base of the auricle.

The pallio-visceral nervous complex in *Ariolimax* (Pl. VII., Fig. 18) does not differ essentially from that of the slug just described. The left mantle and pallial nerve (2) enters the pallial complex close to the rectum. The visceral nerve (*vn*) is given off dorsal and a little to the left on the median protuberance of the ganglion. The nerve takes a posterior direction along the cephalic aorta for a distance of about 3.5 cm., when it divides in two, one branch continuing in the posterior direction along the posterior aorta to the liver and the intestines, the other turning dorsally towards the kidney. Here the nerve bifurcates, both branches entering the kidney at its ventral side, a little to the right of the exit of the aorta through that organ. By aid of a strong dissecting lens a filament from one of these renal nerves can be followed on to the ventriculo-aortic junction. I was not able to trace any nerve branch to the auricle, although stimulation of the visceral nerve shows that nerve fibers enter the heart of this slug both at the aortic and the auricular ends. The dissection reveals no ganglion at the point on the visceral nerve where the reno-cardiac nerve is given off, corresponding to that in *Limax*.

The nervous system of *Helix* (Pl. VII., Fig. 19) resembles that of the slugs very closely. The pallial ganglion gives rise to four nerves, the lateral pair (1, 4) entering the pallial complex and the mantle. The right member of the median pair (2) goes to the pallial complex, but it cannot be traced to the auricle or the kidney, and stimulation of the nerve does not affect the heart. The left member (3) of the median pair is the visceral nerve proper. On its posterior course it gives off a branch to the copulatory organs (5), and a little further posterior another small branch which probably enters the lung (6). At the point of origin of this branch there is a slight ganglionic swelling on the nerve. At the level of the ventricle the nerve bifurcates, one branch continuing in the posterior direction into the reproductive gland (9). The other branch (7) turns to the right and before entering the kidney gives off a filament (8) which takes a posterior direction, probably reaching the stomach and the intestine. The nerve can be followed into the kidney without any difficulty, but I was not able to trace any of its branches through the kidney and into the auricle, as Ransom has described and figured it, although I have physiological evidence that such connection is made. Before the nerve enters the kidney a small filament is given off to the aorta and the pericardium, but I could not trace it on to the ventricular musculature, although the physiological evidence is conclusive that nerve fibers enter the ventricle at the aortic end.

8. *The Cephalopods.* — Nerves to the cardiac apparatus of the cephalopod molluscs have been described by Chéron (1866), Fredericq (1878), and Ransom (1884). Chéron worked on *Eledone*, *Octopus*, *Sepia* and *Loligo*. In the *Octopoda* four nerves are given off from the sub-œsophageal or pleuro-visceral ganglion. Two of these nerves pass to the stellate ganglia in the mantle. The median pair of nerves takes a posterior direction on the ventral surface of the liver and innervates the rectum, the cardiac apparatus and the gills. At the level of the auricles is found a small ganglion on each nerve, and from these ganglia nerves are given off to the auricles and the systemic ventricle. Further on their course each nerve sends a branch to the small ganglion situated on the gill ventricle, while the main nerve trunk enters the large ganglion at the base of the gill. According to Chéron the arrangement is the same in the Decapoda, with the exception of the innervation of the auricles and the systemic ventricle. Chéron describes a commissure between the two visceral nerves a short distance in front of the systemic ventricle. At the junctions of the commissure with the visceral nerves are found ganglionic swellings, which he considers homologous with the ganglia on the visceral nerves of the Octopoda which innervate the auricles and the systemic ventricle. Fredericq and Ransom worked on *Octopus*, but apart from their physiological results they add nothing to the anatomy of the cardiac nerves as given by Chéron. Neither of these investigators make any mention of a commissure between the two visceral nerves similar to that described by Chéron for the decapods, but Fuchs (1895) figures such a commissure in *Octopus*.

My own work was done on *Octopus punctatus*, *Loligo pealii* and *Ommastrephes illecebrosa*. The innervation of the cardiac apparatus in *Octopus* I found in all essentials the same as described by the authors just referred to, and the reader is referred to the figures given by them. In *Loligo* I found some relations not described by Chéron, and the cardiac innervation of this cephalopod will therefore be described with some detail. From the median lobe of the pleural ganglion (Pl. VII., Fig. 20) proceeds a stout nerve, which as it penetrates the cranial cartilage separates into two flattened branches running close to each other in a posterior and ventral direction through the liver, and on reaching its ventral surface they pass posteriorly between the liver, and the viscero-pericardial sac. At this point in their course a small branch is given off from each nerve to the cephalic vena cava, which lies just ventral to the nerves. The two branches unite into one (11) which enters the vein, branching out anteriorly and posteriorly in its muscular walls. A similar nerve to the vena cava is described by Chéron for *Eledone*, *Sepia* and *Loligo*, but he figures it as coming off from the pleural or pallial ganglion a little anterior to the point of origin of the visceral nerves. At the level of the anus the two visceral nerves diverge, taking a more ventral direction and half encircling the vena cava. The circle is made complete by branches given off from each nerve to the rectum and the ejaculatory duct of the ink gland and a commissure between the two sets of nerves (1, 2, 3). The commissure passes between the vena cava and the rectum. One of the branches from the left nerve (2) can be followed in the viscero-pericardial sac near the median line almost to the posterior end of the body cavity. On its course numerous branches are given off to this visceral envelop, but none of these can be traced to any of the cardiac organs. The visceral nerves continue posteriorly close to the vena cava, and at the level of the ink gland, to which branches are given off (4), they take a lateral and dorsal direction, passing dorsal to the auricles and the branchial sinuses to a ganglion at the base of the gill (*bg.*). Before the nerve reaches this ganglion, that is, about 3 mm. central to it, a branch (6) is given off to a small ganglion situated dorso-median on the gill ventricle near the junction of the ventricle with the afferent gill vein (9). From this ganglion a small nerve passes on to the dorsal surface of the auricle (7). At the point of entrance of the nerve into the auricle a small ganglionic enlargement can be made out on the nerve (10). There is some individual variation at this point. Instead of one branch from the visceral nerve to the ganglion on the gill ventricle there are sometimes two or three, one of which usually proceeds from the gill ganglion. A branch may also run past the ganglion on the gill ventricle and join the branch from this ganglion to the auricle. I was not able to follow the nerves that enter the auricles on to the systemic ventricle. The ganglion at the base of the gill measures 1 mm. in diameter in the largest specimens and the ganglia on the gill ventricle and the auricle are only one third as large, so that it is difficult to locate them by dissection, especially as they are imbedded in the connective tissue that envelops these organs. From the gill ganglion a small branch passes to the retractor muscle of the gill (8). In the male a branch from the left visceral nerve innervates the penis (5). At the level where the branches are given off to the ink gland (4) commissural fibers extend across from one visceral nerve to the other. This commissure would correspond to that described by Chéron for the decapods. If branches are given off to the systemic ventricle by the visceral nerves at this point as physiological experiments indicate, and as Chéron found to be the case, the systemic heart of *Loligo* has a double nervous supply, as nerves unquestionably pass to the auricles along with the nerve or nerves to the ganglia on the gill ventricles. Chéron figures the ganglia which supply the nerves to the gill ventricles as situated on the trunks of the visceral nerves, and not on the ventricles themselves, as I found to be the case in *Loligo.*

The dissection of the smaller branches of the nerves in *Loligo* is very difficult, because the nerves, the connective tissue and the muscular tissue have in life very nearly the same color and transparancy ; and in addition, minute arteries, which are hardly distinguishable from the nerves, run in the larger nerve trunks and ramify with the smaller nerve branches.

The specimens of *Ommastrephes illecebrosa* obtained at Woods Holl are more than twice the size of *Loligo.* The species of *Ommastrephes* on the coast of California (*O. californica*) attains a length of from 3 to 3½ feet, but the only specimen of this large species at my disposal was so badly preserved that the finer branches of the nerves could not be told apart from the smaller arteries. The main course of the visceral nerves in *Ommastrephes* (Fig. 21) is the same as in *Loligo*. The commissure (*vc.*) between the two visceral nerves a little anterior to their lateral divergence from the vena cava to enter the gills, is very distinct. The commissure gives rise to a nerve (2) which follows the vena cava in a posterior direction to enter the ventricle. The nerve divides and anastomoses extensively on its course along the vena cava and sends numerous branches to the renal veins. In *Loligo* Chéron figures a nerve as passing to the auricles from either visceral nerve at the level of the commissure in a manner similar to that in *Octopus*. In *Ommastrephes* there is no distinct ganglionic enlargement at the junctions of the commissure with the visceral nerves. The nerve passing to the heart takes its origin from the commissure and not from the visceral nerves directly, and this nerve enters the ventricle, not the auricle.

At the level of the auricles each visceral nerve bifurcates, the smaller branch of each (3, 4) taking a median direction on the ventral and posterior surface of the systemic ventricle. Near the origin of the posterior artery these branches are connected by a tiny commissure, after which they unite into one nerve-trunk (10). This follows the posterior aorta for a little distance to finally enter the reproductive organs. There is a slight ganglionic enlargement at the place of bifurcation.

The branches to the gill ventricles and the auricles (5) leave the visceral nerves close together a little distance before these nerves enter the gills. The nerve to the gill heart (6) runs posteriorly along afferent gill sinus and penetrates the substance of the gill heart at its junction with this sinus. The auricular nerves are very slender. In some specimens they are given off, not from the visceral nerves directly, but from the branch to the gill ventricles. There is a distinct ganglionic enlargement on the visceral nerves at the point of origin of the nerves to the gill ventricles.

This ventral visceral nervous complex is connected with the cerebro-gastric nervous system by a commissure (8) from the left visceral nerve to the gastric ganglion (*g.*). This nervous connection has not, to my knowledge, been noted before in the cephalopods. I was unable to make it out with a certainty in *Loligo* owing to the difficulty of distinguishing between the smaller arteries and the finer nerve-branches.

The systemic heart of *Ommastrephes* (and in all probability also *Loligo*) is thus furnished with a double nervous supply similar to that of the gasteropod molluscs, fibers from the visceral nervous complex entering the systemic heart both at the auricular and at the aortic ends. The presence of the communicating branch between the gastric ganglion and the visceral nerves is of interest in view of the statement by Bottazzi and Enriques (1901) that the cerebro-gastric commissure in the œsophagus contains accelerator fibers to the systemic heart (*Octopus*).

9. *The Decapod Crustaceans.* — Considerable work has been done on the innervation of the heart in the decapod crustaceans. Lemoine (1868) describes, in the crab, a branch to the anterior and dorsal surface of the heart from the gastric nerve. The gastric nerve makes direct connection with the cerebral ganglia and the œsophageal commissures, but not with the thoracic ganglion. The existence of this cardiac nerve or nerve of Lemoine was confirmed on anatomical as well as physiological grounds by Young (1878, 1879), Plateau (1878, 1880), and Moquart (1883). Plateau states that "l'existence du *nerf cardiaque* de Lemoine est incontestable." Both Young and Plateau conclude that this cerebro-cardiac nerve is accelerator in function, and that in addition to this accelerator nerve the heart is also supplied with inhibitory nerves from the thoracic ganglion. Important modifications and additions to these results have been made by Jolyet and Viallanes (1892) and by Connant and Clarke (1896). Jolyet and Viallanes found that in the crab (*Carcinus*) both the accelerator and inhibitory centers for the heart are situated in the thoracic ganglion, and that when the cerebro-thoracic commissures are severed, stimulation of the cerebral ganglia or the gastric nerve has no effect on the heart. Nor could they find the "nerve of Lemoine" anatomically. In the pericardium they found a plexus of nerve cells and nerve fibers, from which proceed three nerves on either side in a ventral direction towards the thoracic ganglion, but the connection of these nerves with the ganglion was not made out. This pericardial nerve plexus had previously been described by Dogiel (1878). The results of Jolyet and Viallanes were confirmed by Connant and Clarke on the crab (*Callinectes*). They were equally unable to obtain any anatomical or physiological evidence of the existence of the "nerve of Lemoine," but they traced the connection between the thoracic ganglion and the six nerves which Jolyet and Viallanes had described as entering the pericardial nerve plexus. These

cardiac nerves take their origin dorsally on the thoracic ganglion close to the origin of the recurrent cutaneous nerve, the nerve to the third maxilliped, and the nerve to the first ambulatory appendage respectively. The nerves that reach the heart in the trunk of the recurrent cutaneous nerve, that is, the anterior pair of cardiac nerves, were found to be inhibitory in function, the two posterior pairs to be accelerator. Bottazzi (1901) has confirmed the results of Jolyet and Viallanes and Connant and Clarke as regards the presence in the thoracic ganglion of both accelerator and inhibitory centers to the heart.

Nerve cells in the walls of the crustacean heart have been described by Berger (1876), Deszö (1878), Young (1878), Dogiel (1877, 1894), Plateau (1880), and Pagoschwa (1890). Deszö states that the ganglion cells are especially abundant in the posterior half of the dorsal wall.

The results of Jolyet and Viallanes and of Connant and Clarke on the crab appeared to me so conclusive that I did not take the time to work out the cardiac nerves in the available material of this group (*Cancer*, *Brachynotus*, *Epialtus*), but instead I confined the anatomical work to the large spiny lobster (*Palinurus*), and it may be stated at the outset that the cardiac nerves in this decapod have nearly the same relations as given by Connant and Clarke for *Callinectes*. The dorsal side of the large thoracic ganglion gives rise to six pairs of small nerves (Pl. VIII., Fig. 22, 1 to 6). The anterior pair takes its origin near the roots of the nerves to the third maxilliped. On leaving the ganglion the nerves run alongside (but not in the same sheath as) the recurrent cutaneous nerves for some distance in an anterior, lateral and dorsal direction. The recurrent nerves turn posteriorly after passing around the anterior processes of the endophragmal skeleton, but their smaller companions (1) pass through the foramina between the two anterior endostermites. Reaching the inner surface of the endoskeleton at this point the nerves take a dorso-lateral direction ventral to the anterior flexor muscles of the abdomen, and on reaching the point of attachment of the extensor muscles the nerves pass posteriorly on these muscles and finally ramify in them posterior to the level of the pericardial cavity. At the level of the pericardial cavity each nerve gives off a branch (7) to the pericardium and the heart, the slender filaments reaching the dorsal and posterior side of the heart along the suspensory ligaments. The nerves that are given off from the thoracic ganglion near the roots of the first ambulatory nerves (2) take a dorsal direction through the opening between the second and the third endosternites and pass ventral and lateral to a greater bulk of the flexor muscles than is the case of the anterior pair. On reaching the extensor muscles the nerves run posteriorly on their surface, ventral to the anterior nerves, and at the level of the anterior end of the heart each nerve gives off a small branch (8) to the plexus of arteries and suspensory ligaments at the anterior end of the pericardial cavity. In very large specimens the nerves can be followed along the suspensory ligaments to the dorsal side of the heart.

The other nerves from the dorsal surface of the thoracic ganglion (3 to 6) turn dorsally through the openings of the subsequent endosternites and ramify, with the arteries, on the flexor and extensor muscles that are situated dorso-laterally in the thoracic cavity. I could not trace any of their branches to the heart, nor does stimulation of these nerves effect the heart. That the two anterior pairs of nerves really send fibers to the heart is further shown by stimulation of these nerves. The anterior pair contains inhibitory, the second pair accelerator fibers. In *Callinectes* Connant and Clarke found that the inhibitory fibers reach the heart in the recurrent cutaneous nerves (*rcn.*). This is not the case in *Palinurus*. Branches from the posterior rami of these nerves can be followed close up to the pericardial cavity, but not on to the heart itself, or on to the suspensory ligaments, and in no instance did stimulation of these nerves effect the heart. The course of the cardiac nerves in the plexus of suspensory ligaments and small arteries in and surrounding the pericardiac cavity is not easily made out, because the nerves, the arteries and ligaments have nearly the same color and transparency.

10. *The Arachnids.* — To the recent paper on the innervation of the heart of *Limulus* by Patten and Redenbaugh (1899) I have only one item to add, viz., the connection of the inhibitory nerves with the heart. The heart and the heart-nerves of this interesting arthropod are shown in Fig. 23. The ganglion cells

and the main nerve-trunks are confined to the dorsal side of the heart. The large nerve-trunk on the dorso-median side of the heart is in reality an elongated ganglion, being made up of nerve-fibers and ganglion cells. This nerve-cord or ganglion extends the whole length of the heart, but it is largest in the fourth, fifth and sixth segments, tapering thence both in the anterior and the posterior direction. There are relatively few ganglion cells in the nerve-cord of the first and second segments. Besides this elongated ganglion on the dorso-median side of the heart there are two nerves running parallel with it and lateral to the ostia. These are the lateral nerves. The nerves branch and anastomose extensively, especially in the middle region of the heart. In large specimens the nerves can be followed with the naked eye up to the first, and posteriorly to the last segment, but they are stoutest in the fourth to the sixth segments. There appears to be no ganglion cells on the course of these nerves. A very complex system of connectives extends between the median nerve-cord and the lateral nerves. On the whole, one pair of these connectives are given off from the median nerve-cord at the level of each pair of ostia, the connectives being the largest in the fourth, fifth and sixth segments. The connectives usually branch extensively on the dorsal side of the heart before joining the lateral nerves. The median nerve-cord, the lateral nerves as well as the main connecting branches are large enough to be easily made out with the naked eye in the living heart.

The nervous complex on the dorsal side of the heart is partly covered by the elastic connective tissue fibers, but these can be removed without any injury to the nervous elements. Both the nerve-cord and the nerves are, on the other hand, separated from the heart-muscle by the basement membrane. This allows a complete removal of the nervous complex without any injury to the heart-muscle.

The connections between this cardiac nervous complex and the brain and the abdominal ganglia are shown in Figs. 24 and 25. These connections have been carefully worked out by Patten and Redenbaugh, but these investigators failed to find any connection between the hæmal nerves given off from the posterior end of the brain (7, 8) and the nerve-cord on the heart. These nerves do make such a connection, as I have represented in Fig. 24. This is also borne out by the physiological experiments.

From the dorsal side of the brain corresponding to each pair of nerves to the ambulatory appendages is given off a pair of nerves which take a dorsal direction to innervate the integument, the viscera, and dorsal musculatures. These nerves are evidently homologous to the nerves occupying a similar relation to the thoracic ganglion in *Palinurus* (Fig. *22*, 1–6). The anterior pair of nerves from each abdominal ganglion takes a similar course and make connections similar to those from the dorsal side of the brain. As these nerves go to innervate organs lying dorsal or hæmal to the level of the central nervous system, Patten and Redenbaugh call them hæmal nerves in contradistinction to the nerves to the ambulatory appendages and the gills which are designated as neural nerves.

The hæmal nerves from the abdominal chain of ganglia (Fig. *25*, 9–13) takes a dorsal and posterior direction and after giving off their fibers to the intestine and the integument, penetrate the pericardial cavity, each sending a small fillament along the dorsal wall of the pericardial cavity to unite with the nerve-cord on the heart approximately opposite the fourth to the eight pairs of ostia. These cardiac branches are, with the exception of those from the ninth and the tenth nerves, so tiny that they are not readily made out in the living tissue. Prior to the entrance of these nerves into the dorsal pericardium to connect with the nerve-cord each nerve sends a communicating branch to the nerve which runs parallel to the heart in either angle of the pericardial cavity. These nerves are designated as pericardial nerves by Patten and Redenbaugh.

Of the hæmal nerves given off from the brain the only ones that I was able to trace to the heart are the last two pairs (Fig. *25*, 7, 8). The cardiac branches of these nerves unite in one common trunk before reaching the pericardial cavity. The main branches of this large nerve go to make up the pericardial nerves and to innervate the large inter-tergal muscle, which lies dorsal and lateral to the heart in this region. The branches that pass to the epidermis in the median line connect with the nerve-cord on the heart in the manner shown in Fig. 24. There is considerable individual variations as to the exact place of connection with the nerve-cord, in some specimens there appears to be connections only at the level of the second pair of ostia, in others the connection is made in the middle of the third segment, while in some the main, if not the only, connection is the one just behind the third pair of ostia.

It is rather difficult to homologize the cardiac innervation of the crustaceans with that of *Limulus*. In the crustacean heart the ganglion cells are not congregated in a single ganglion on the surface of the heart, but scattered throughout the muscle. There appears, however, to be this homology that in the crustacean heart the ganglion cells are massed particularly at the posterior end of the dorsal wall of the heart. In *Palinurus* the cardiac nerves take their origin from the anterior end of the thoracic ganglion, the abdominal ganglia not making any connections with the heart; in *Limulus* the cardiac nerves take their origin from what actually corresponds to the thoracic ganglion and from the abdominal ganglia as well. This difference is probably due to the fact that the *Limulus* heart retains its primitive elongated character while the crustacean heart is very much shortened and confined to a small space in the cephalothorax.

Regarding the innervation of the heart in insects we have the observations of Müller and Brandt (quoted by Kolbe, 1893) that the heart and the aorta is innervated from the second pair of œsophageal ganglia. This cardiac nerve would thus seem to correspond to the "cardiac nerve of Lemoine" in the crustaceans, which, as we have already pointed out, appears not to have any basis in fact. To my knowledge no connection between the abdominal ganglia and the heart analogous to that in *Limulus* has been traced in the insects.

Dogiel (1877) has described ganglion cells on the heart or in close proximity of the heart of the larva of *Corethra plumicornis*. According to Lang (1900) a nerve or nerve-cord on the dorsomedian side of the heart similar to that in *Limulus* has been described in some of the myriapods (*Peripatus*, *Jules*).

11. *The Tunicates.* — Despite numerous researches with the view of finding nerve cells and nerve fibers in the heart of the tunicates the results have been, until a recent date, uniformly

negative; neither nerve cells nor nerves could be found. The uniform negative results were generally taken to indicate that nervous elements are not present in the heart of this interesting group of animals. But Hunter (1902) has recently found nerves and nerve cells in the heart of *Molgula manhattensis.* The nerve cells, mostly of the bipolar type, are most abundant at either end of the heart, situated on the surface of the muscular walls under the pericardium. The same observer has later (1903) found evidence to the effect that the cluster of nerve cells or ganglia at either end of the heart are connected by nerve fibres with the central nervous system.

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Explanation of Figures.

Plates IV–VIII.

Abbreviations.

A, anterior artery.
AC, abdominal commissures.
AOS, aortic sinus.
AU, auricle.
B, brain.
BG, branchial ganglion.
CC, cerebral commissure.
CG, cerebral ganglion.
CPC, cerebro-pedal commissure.
CPG, cerebro-pleural ganglion.
CVC, cephalic vena cava.
CVCO, cerebro-visceral commissure.
G, gastric ganglion.
GI, genital ganglion.
GN, genital nerve.
GV, gill ventricle.
H, heart.
HG, hind gut.
IC, intestino-visceral commissure.
K, kidney.
LA, lateral arteries.
LN, lateral nerves.
LVG, left visceral ganglion.
LVN, left visceral nerve.
MNC, dorso-median nerve-cord.
OC, œsophageal commissure.
OE, œsophageal opening.
OER, œsophageal nerve-cord.
OS, ostia.
PA, posterior aorta.
PAM, posterior adductor muscle.
PC, pedal cord.
PCN, pericardial nerves.
PG, pedal ganglion.
PLG, plural ganglion.
PIC, pleuro-intestinal commissure.
PLC, pleuro-visceral cord.
PN, pallial nerve.
PVG, pleuro-visceral ganglion.
PS, posterior sinus.
R, rectum.
RCN, recurrent nerve.
RV, renal vein.
RVG, right visceral ganglion.
RVN, right visceral nerve.
SA, sternal artery.
SL, suspensory ligaments.
SOG, supraœsophageal ganglion.
SG, subintestinal ganglion.
SUG, supraintestinal ganglion.
SV, systemic ventricle.
THG, thoracic ganglion.
V, ventricle.
VC, visceral commissure.
VG, visceral ganglion.
VN, visceral nerve.

EXPLANATION TO PLATE IV.

FIG. 1. Mytilus californianus. Dorsal view. 1, posterior mantle nerve; 2, branchial nerve; 3, nerve to posterior adductor muscle; 4, nerves to dorsal body wall and pericardium; 5, nerves to base of auricles; 6, nerves to body wall and pericardium; 7, nerves passing posteriorly in the dorsal body wall; 8, 9, 10, ganglia on nerves 4; 11, nerves from the cerebro-visceral commissure to the kidneys; 12, nerves from the cerebro-visceral commissures to the adductor muscles of the foot and the byssus.

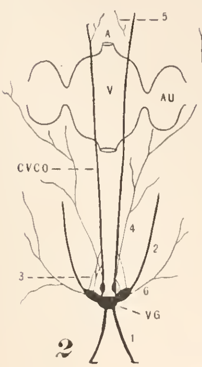
FIG. 2. Mya arenaria. Ventral view. 1, nerve to mantle and siphon; 2, branchial nerve; 3, nerve plexus on the ventral surface of the kidney between the cerebro-visceral commissures and the visceral ganglion; 4, nerves to kidney (and heart); 5, nerves from the cerebro-visceral commissure to the reproductive glands; 6, nerves to kidney.

FIG. 3. Mya arenaria. Lateral view. 1, nerve to mantle and siphon; 2, nerve to posterior adductor muscle, dorsal body wall and pericardium; 3, branch to kid, ney; 4, branch to posterior aorta and hind gut.

FIG. 4. Tapes staminea. Ventral view. 1, posterior mantle nerves; 2, siphonal nerves; 3, nerves to posterior adductor muscle; 4, nerves to osphradium and gill; 5, reno-cardiac nerves.

FIG. 5. Platydon cancellatus. Ventral view. 1, nerves to siphon and mantle; 2, nerves to osphradium and gill; 3, nerves to renal organ and heart; 4, ganglia at the base of the auricles.

FIG. 6. Cryptochiton stelleri. Ventral view. 1, nerves from pleuro-visceral cords to auricles; 2, nerves that can be followed to the point of attachment of the auricles to the body wall; 3, 4, connectives between the nerve cords.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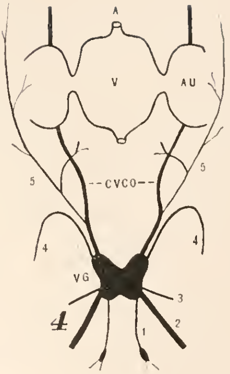
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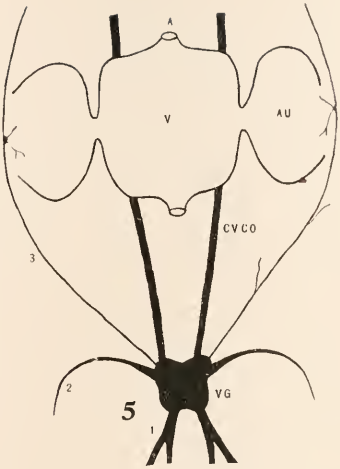
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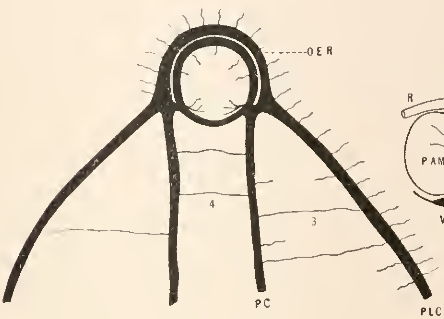
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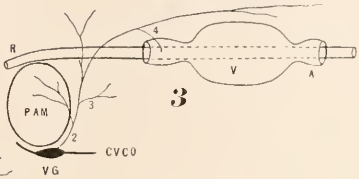
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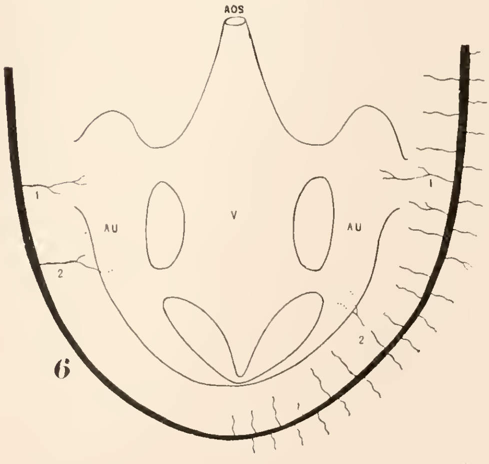
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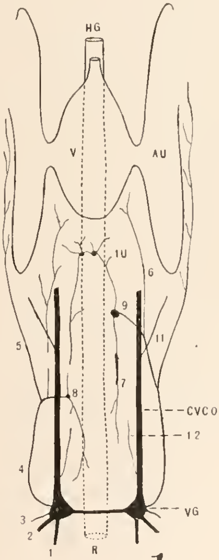
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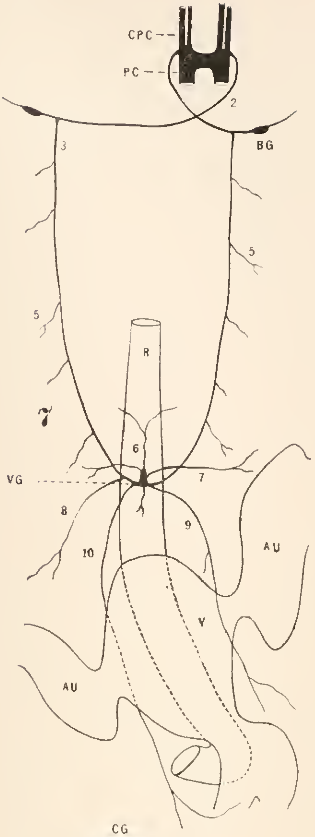
EXPLANATION OF PLATE V.

FIG. 7. Haliotis cracherodii. Dorsal view. 1, 2, pleuro-visceral commissure ; 3, 4, nerves to the visceral ganglia ; 5, nerves to floor of pallial cavity and efferent gill sinus ; 6, nerve to rectum ; 7, 8, nerves to base of auricles ; 9, nerve to dorsal pericardium ; 10, nerve to ventral wall of paricardium and aorta.

FIG. 8. Lucapina crenulata. Dorsal view. 2, nerve to afferent gill sinus ; 3, 4, 5, nerves to the wall of paricardial cavity ; 6, nerve to rectum ; 7, nerves to renal organs ; 8, nerve to mantle fissure ; 9, nerve to efferent gill sinus ; 10, nerve to osphradium and gill.

FIG. 9. Natica lewissii. Dorsal view. 1, nerve to dorsum ; 2, nerve to floor of pallial cavity ; 3, nerve to osphradium ; 4, nerve to gill ; 5, commissure between branchial and left visceral ganglia ; 6, nerve to pericardium, heart and kidney ; 7, ganglia on commissure ; 8, genital nerve ; 9, nerve to paricardium, heart and kidney.

FIG. 10. Sycotypus canaliculatus. Dorsal view. 1, nerve to floor of pallial cavity ; 2, nerve to pericardium and auricle ; 3, nerve to ventricular ganglion ; 4, nerve to liver and genital organs ; 5, nerve to ventricular ganglion ; 7, renal nerve ; 8, ganglion on ventriculo-aortic junction ; 9, ventricular nerves ; 10, pleuro-branchial commissure ; 11, nerve to hind-gut.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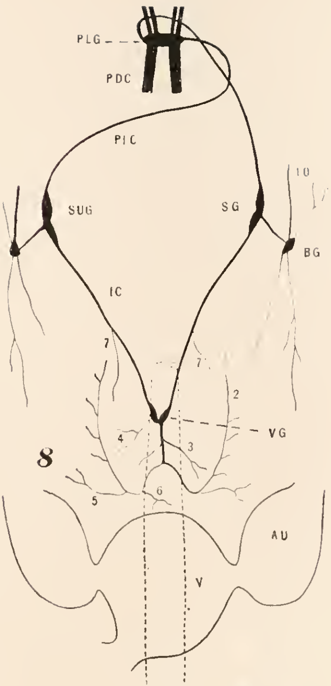
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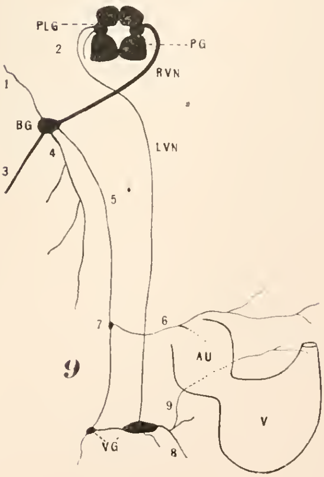
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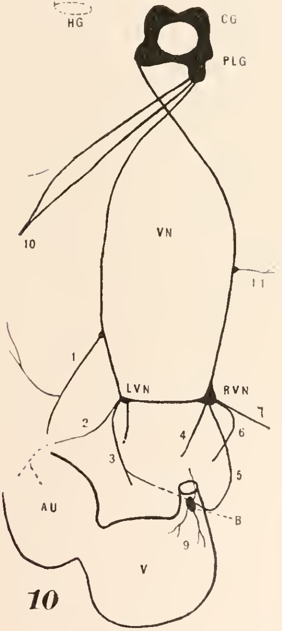
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EXPLANATION OF PLATE VI.

FIG. 11. Aplysia californica. Dorsal view. 1, nerve to osphradium ; 2, branch to roof of gill chamber ; 3, nerve to viscera ; 4, nerve to gill ; 5, nerve to dorsum of the anal region ; 6, nerve to liver, ventricle and reproductive glands ; 7, nerve to pericardium, kidney and auricle ; 8, branches to kidney ; 9, branch to auricle ; 10, branch to liver ; 11, branch to the reproductive gland ; 12, branch to aortic sinus ; 13, nerve connecting right pedal and visceral ganglia.

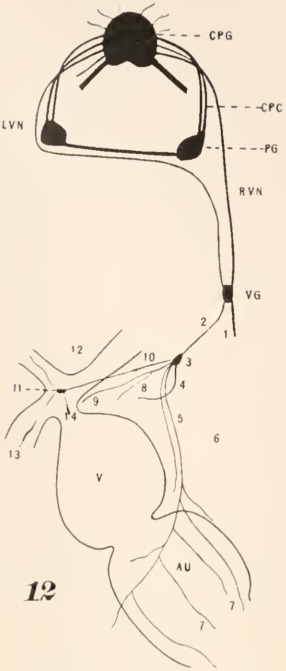
FIG. 12. Pleurobranchæa californica. Dorsal view. 1, nerve to osphradium and gill ; 2, nerve to viscera ; 3, accessory visceral ganglion ; 4, genital nerve ; 5, nerve to pericardium and auricle ; 6, 8, branches to pericardium ; 7, nerves in the walls of the auricle ; 9, branch pericardium and aortic sinus ; 10, branch to aortic sinus ; 11, ganglion on aortic sinus ; 12, branch to the lateral artery ; 13, branch to posterior artery ; 14, branch to aortic sinus, probably entering the ventricle.

FIG. 13. Bulla globosa. Dorsal view. 1, ganglia on left visceral nerve; 2, nerve to dorsum of neck ; 3, commissure between the right and the accessory visceral ganglia ; 4, commissure between the left and the accessory visceral ganglia ; 5, nerve to osphradium, gill and roof of gill chamber ; 6, nerve to inferior pallium and dorsum ; 7, accessory visceral ganglion ; 8, nerve to viscera ; 9, ganglion on visceral nerve ; 10, genital nerve ; 11, branch to aortic sinus and aorta ; 12, nerve to pericardium, kidney and auricle ; 13, branch to auricle ; 14, branch to kidney.

FIG. 14. Montereina nobilis. Dorsal view. 1, branch of visceral nerve passing ventral to the hind gut ; 2, branch to ganglion on the aortic sinus ; 3, branch to pericardium ; 4, branch to base of auricle ; 5, branch to gill ; 6, ganglion on the aortic sinus.

FIG. 15. Triopha carpenteri. Dorsal view. 1, 2, nerves to the mantle ; 4, left visceral nerve ; 6, right visceral nerve ; 7, nerve to genital ganglion ; 10, branch to anterior aorta ; 11, branch to gill and auricle.

FIG. 16. Triopha grandis. Dorsal view. 1 and 2, nerves to dorsum and gill ; 3, reno-cardiac nerve ; 5, ganglion on the reno-cardiac nerve ; 7, nerve aorta and ventricle ; 8, auricular nerves ; 9, ganglia at the base of the gill ; 10, optic nerve.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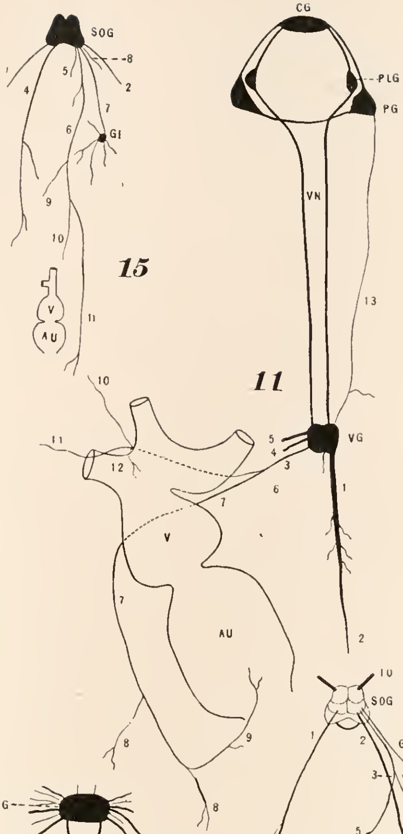
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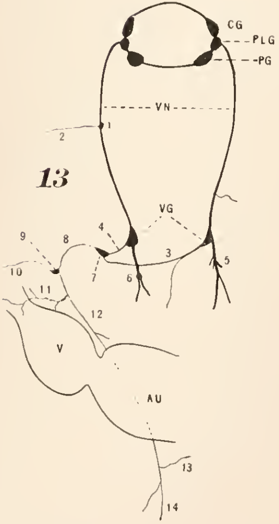
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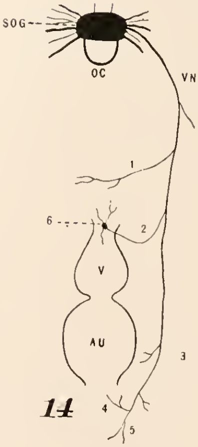
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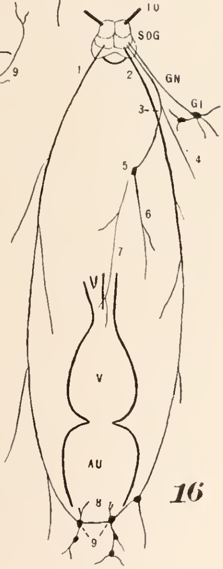
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EXPLANATION OF PLATE VII.

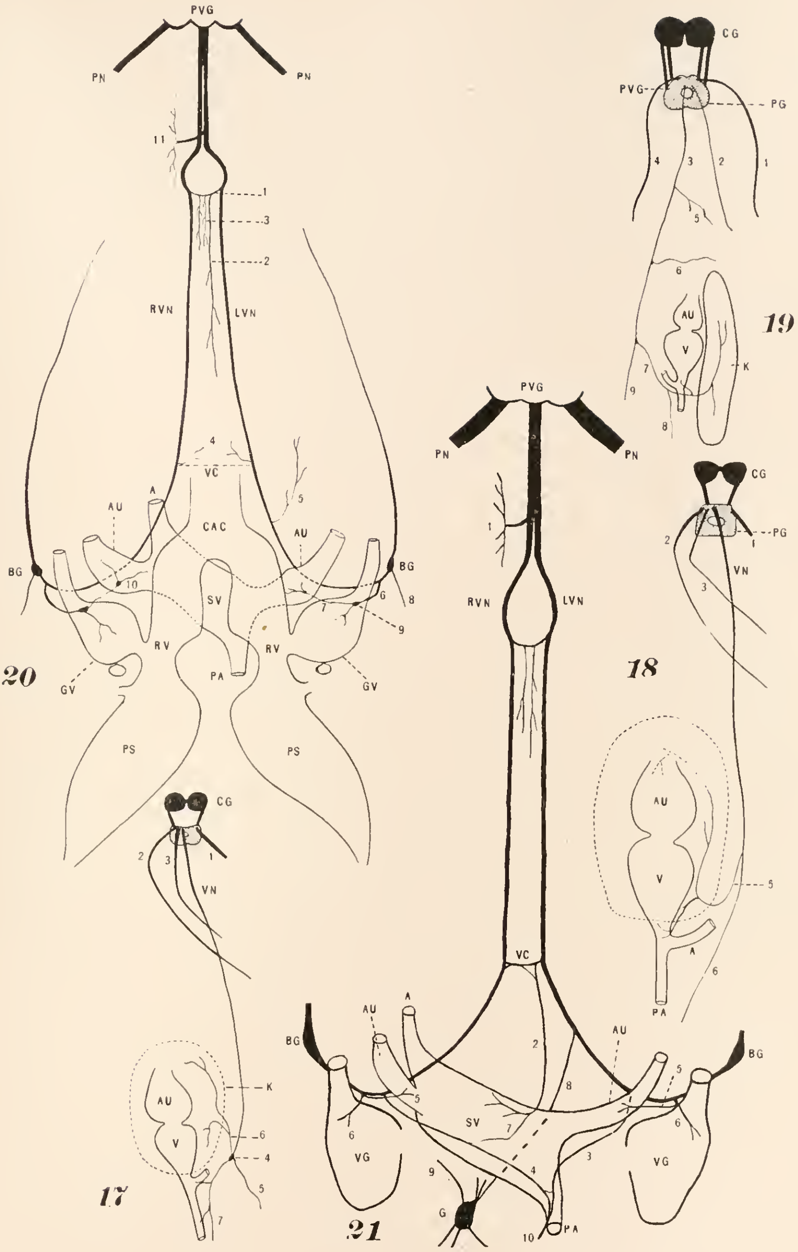
FIG. 17. Limax maximus. Dorsal view. 1, 2, 3, nerves to mantle and pallial cavity; 4, visceral ganglion; 5, genital nerve; 6, reno-cardiac nerve; 7, nerve to liver and intestine.

FIG. 18. Ariolimax columbianus. Dorsal view. 1, 2, 3, nerves to mantle and pallial cavity; 5, reno-cardiac nerve; 6, nerve to intestine and liver.

FIG. 19. Helix (Arionta) dupetitouarsi. Dorsal view. 1, 4, nerves to mantle; 2, nerve to pallial cavity; 3, visceral nerve; 5, branch to genital organs; 6, branch to lung; 7, reno-cardiac nerve.

FIG. 20. Loligo pealii. Ventral view. 1, commissure between the visceral nerves ventral to the vena cava; 2, nerve to the viscero-pericardial envelop; 3, nerves to rectum and duct of the ink gland; 4, nerves to ink gland; 5, nerve to penis; 6, cardiac nerve; 7, auricular nerves; 8, nerves to adductor muscles on the gills; 9, ganglia on gill ventricles; 10, ganglia on auricles; 11, nerves to vena cava.

FIG. 21. Ommastrephes illecebrosa. Ventral view. 1, nerve to vena cava; 2, nerve to ventricle; 3 and 4, nerves to genitalia and osphradium; 5, nerves to auricles; 6, nerves to the gill ventricles; 7, anastomosing branch between nerve 4 and the ventricular nerve; 8, nerve connecting the left visceral nerve with the gastric ganglion; 9, commissure between the brain and the gastric ganglion.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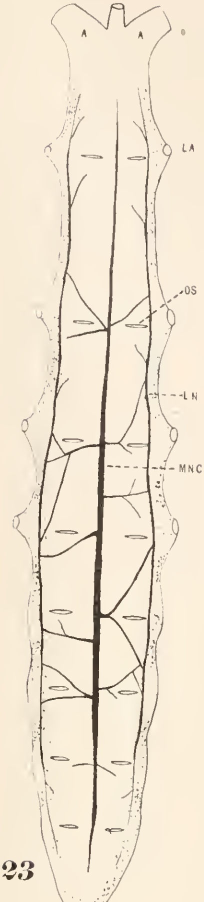
EXPLANATION OF PLATE VIII.

FIG. 22. Palinurus sp. Dorsal view, heart displaced posteriorly. I–V, nerves to corresponding ambulatory appendages; *3m*, nerve to third maxilliped; 1, 2, nerves to adductor muscles and heart; 3–6, nerves ramifying in the arterial plexus on the adductor muscles; 7, 8, cardiac nerves.

FIG. 23. Heart and heart-nerves of Limulus polyphemus. Dorsal view. *a*, anterior artery; *la*, lateral arteries; *ln*, lateral nerves; *mnc*, median nerve-cord; *os*, ostia.

FIG. 24. Heart of Limulus. Dorsal view. Showing the connection of the ventral with the cardiac nervous system. *mnc*, dorso-median nerve-cord on the heart; *pn*, pericardial nerves; 7, 8, branches from the two posterior hæmal nerves from the brain; 9–13, branches from the hæmal nerves of the abdominal ganglia.

FIG. 25. Brain and abdominal ganglia of Limulus. Dorsal view. I–V, nerves to corresponding ambulatory appendages; 2–8, hæmal nerves from the brain. The nerves 7 and 8 carry the inhibitory fibers to the heart. 9–13, hæmal nerves from the thoracic ganglia, carrying accelerator fibers to the heart.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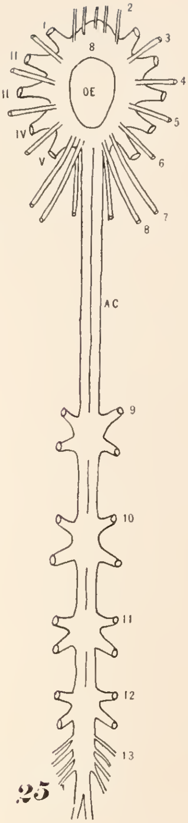
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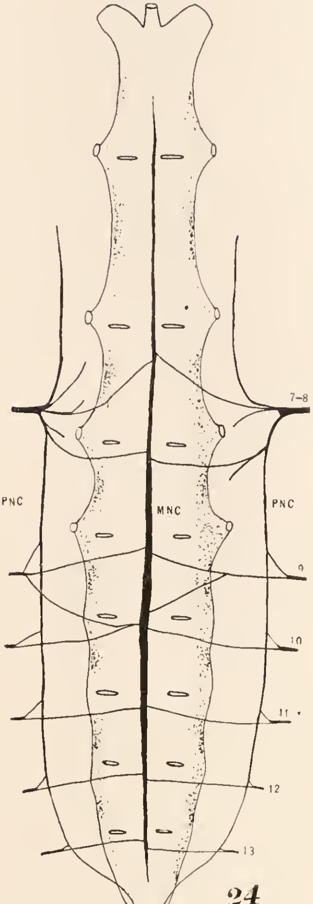
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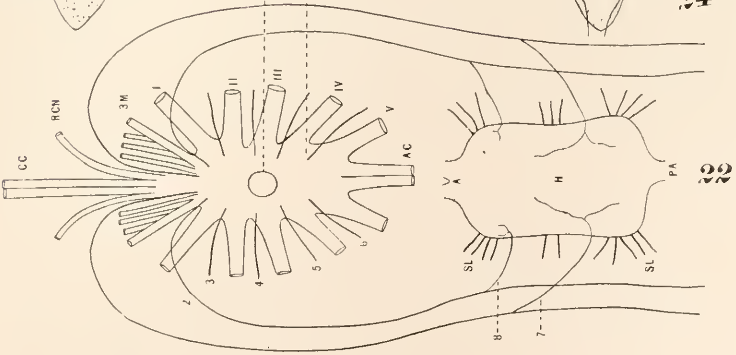
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22

THE SPERMATOGENESIS OF THE SPIDER.

With Plates IX. and X.

LOUISE BAIRD WALLACE.

About four years ago, when the present work was undertaken, several investigators had described a peculiar chromosome in insect spermatogenesis, but outside of the insects it had not then been reported and it was a matter of considerable interest to discover whether or not it obtained generally in the maturation of the germ cells of the Arthropoda. Since Dr. Montgomery had failed to find it in *Peripatus*, he suggested to me the advisability of trying some higher form. A study of the reproductive organs of *Limulus* was first attempted but these proved unfavorable on account of the minuteness of the cells. The testes of the spider were then examined and were found to be promising material for, as an offset to the rather large number of chromosomes characteristic of the Arachnida, the germ cells are of fairly good size and the peculiar chromosome forms a conspicuous structure of the cell even to a casual observer.

For various reasons the common tube-weaving spider, *Agalena nævia*, has been taken as the basis for work, but a comparative study of a few representatives from the Drassidæ, Attidæ, Lycosidæ, Therididæ and Epeiridæ was found of great value in the interpretation of some points. After the publication of a brief preliminary in the fall of 1900, the problem was laid aside until the fall of 1903, when it was resumed at the University of Pennsylvania uuder the direction of Dr. E. G. Conklin. It gives me pleasure to cordially express my indebtedness to Dr. Conklin for his ever ready encouragement and helpful criticism throughout the year.

METHOD.

By almost common consent the strong solutions of Flemming's chromo-aceto-osmic mixture and Hermann's platino-aceto-osmic mixture have been chosen as giving the finest fixation for work in spermatogenesis and while I have used a number of different fluids, I can add my testimony to the superiority of the preparations when the tissues have been treated with one or the other of these two solutions. In order to check as far as possible any changes in the cells which might take place after the death of the animal the spiders were decapitated and the testes rapidly dissected out while the visceral mass was immersed in the fixing fluid. The paired, slightly convoluted tubular organs lie embedded in the liver and contain no pigment so that in immature spiders it is not always an easy matter to distinguish them from the spinning glands which lie ventral to them. As in many other forms, a cross section of the spider testis shows a more or less complete series of developmental stages, with the spermatogonia at the periphery and the cells gradually increasing in maturity toward the lumen. In the breeding season the lumen of the tube is found to be full of mature spermatozoa. Ripe spermatozoa were also taken from the pedipals and studied in smear preparations, sometimes stained *intra vitam* and sometimes fixed, before staining, by heating at the boiling point. Of staining methods, Heidenhain's iron-hæmatoxylin and Hermann's triple stain yielded the finest results.

The Spermatogonia.

The only comparatively recent work upon spider spermatogenesis of which I have any knowledge is that of J. Wagner. Unfortuately his paper is not accessible to me, but from his preliminary report and from several full reviews, I have been able to learn enough of his results to know that they differ widely from mine. He mentions the fact that the spermatogonia are larger than the spermatocytes even at the close of the growth period of the latter. This is true of the spermatogonia of the last generation, but in the early generations the cells are smaller. During the rest stage, when the chromosomes become granular and the chromatin granules are distributed along the linin reticulum, two chromosomes are conspicuous because they take no part in this disintegration (Pl. I., Fig. 1). From this point to the formation of the spermatozoön these two chromosomes can be identified by their shape, by their peripheral position and by the fact that they show a strong affinity for safranin, in the Hermann's staining method, while the other chromosomes take the gentian violet excepting in the metaphase and anaphase, when they also take the safranin. By some authors this is supposed to be due to the presence of a greater amount of nucleinic acid at the height of the mitotic division and since the two peculiar chromosomes in the spider show this reaction constantly throughout the maturation processes it seems possible that they are more richly supplied with nucleinic acid than the other chromosomes. This is of special interest when we recall that the nuclei of spermatozoa contain a maximum of phosphorus.

The rod-shaped chromosomes are so numerous that it is difficult to make an accurate estimate of their number but by repeatedly counting those found in a cross section of the equatorial plate I believe the number to be forty (Pl. I., Fig. 2). In such a polar view of the equatorial plate two of the forty chromosomes are found in a peripheral position, thus reducing the number of other chromosomes to thirty-eight. The ordinary mitotic division occurs in which forty daughter chromosomes are carried to each pole, so it is puzzling to know why Wagner should say "Die Kerne der spermatogonien theilen sich nicht nach dem gewöhnlichen schema der Karyokinese aber auch nicht amitotisch." During mitosis the two peripheral chromosomes split longitudinally and are equally distributed to the two poles, just like the other chromosomes (Pl. I., Figs. 3, 4 and 5). During the telophase when the daughter chromosomes become granular and begin to spread out on the linin reticulum — even at the beginning of this disintegration — two are conspicuous because they remain unchanged and in the rest stage these two rods are sharply defined as they lie in the chromatin reticulum. Usually they lie side by side but are sometimes found at a short distance from one another and I am inclined to think that in the latter case they are slightly dislodged by the microtome knife and so become separated (Pl. I., Figs. 6, 7 and 8.)

A word might be said here in regard to the name of the peculiar chromosomes. In a recent paper ('04) Montgomery has suggested the general term heterochromosome to include the nuclear elements which have been described under various names, and he then divides them into two groups, according to their origin, as follows: (1) "The accessory chromosome"

(McClung), unpaired in the spermatogonia, as found in the Orthoptera. This would include the "chromosome spécial," described by de Sinéty; (2) the "chromatin nucleoli" (Montgomery), paired in the spermatogonia as found in *Euchistus.* This would include the "small chromosome" of Anasa, described by Paulmier. Such a classification does not apply in the spider, where the heterochromosomes are similar in appearance and behavior to those described by McClung as accessory chromosomes, but differ from them in arising as a double element. Sutton's work on *Brachystola* plainly shows the accessory chromosome to arise as a single element in the spermatogonia. The heterochromosomes of the spider, so far as their double origin is concerned, resemble the chromatin nucleoli of *Euchistus* and the "small chromosomes" of *Anasa*, but are otherwise different from them. In view of these facts, McClung's term is employed in this paper, and as the accessory chromosomes never fuse together, but only lie in close contact, the term will be used in the plural.

PRIMARY AND SECONDARY SPERMATOCYTES.

Early in the prophase of the primary spermatocyte the chromatin is finely distributed on a delicate linin reticulum and the accessory chromosomes retain their individuality (Pl. I., Fig. 9). This stage I believe to be comparable to the synapsis, for at this time, or possibly earlier, the pseudo-reduction occurs. In none of my best preparations do I find a massing of the chromatin at one side of the nuclear cavity. Such a massing is found in poorly fixed material, but even then it occurs only in the later spireme stages. In his paper on *Peripatus* Montgomery has asserted that in synapsis is accomplished an end-to-end union, in pairs, of entire chromosomes, and that this numerical reduction occurs during the retrogressive stages of the telophase of the last spermatogonic division. Blackman, in his work upon Myriapods, in discussing this point, adds: "It can be stated with the greatest certainty that pseudo-reduction occurs during the telophase of the last spermatogonium and is completed before the reconstruction of the nuclear membrane." From the synapsis stage, represented in Fig. 9, arises the spireme. Like other workers, I find it impossible to state positively that it is segmented from the first but I think such to be the case, and also that the nineteen loops are connected by a band of linin only. These loops grow thicker and show a longitudinal split gradually increasing in width, while the nucleus becomes more swollen with nuclear sap (Pl. I., Figs. 10–13). At this time a definite polarity can be noted, the blind ends of the loops being directed away from that portion of the cell which contains the greater mass of cytoplasm and the centrosome. The accessory chromosomes lie in the embrace of the free ends of a loop and near the centrosome. In testes nearing maturity, the majority of the cells are in this stage. This is followed by a rather rapid shortening of the loops when they draw down toward what Montgomery has called the distal pole (Pl. I., Figs. 14, 15). In his description of this stage Wagner says: "Der Linin faden (resp. die Reihen der chromatin-körner) bildet Schleifen, die alle gleich lang sind und die gleich Richtung haben: in dieser Weise theilt sich der Linin faden in Stücke von gleichen Länge. Gleichzeitig bildet sich der Nucleolus." While I agree with him in regard to the same general direction of the loops I do not agree with him in his statement that they are equal in length. At this stage the difference in the size of the loops can be plainly seen. This condition favors Montgomery's view of the end-to-end conjugation of chromosomes of like size during synapsis and which has been confirmed by Sutton in his work on *Brachystola*. It seems highly probable, in the light of recent research, that Montgomery's theory in regard to the pairing of paternal and maternal chromosomes during synapsis is the true explanation of the numerical reduction occurring at this time.

In the prophase of the primary spermatocytes, the bend of the loops becomes more acute, while the arms shorten and thicken and the now V-shaped chromosomes, varying in size, are scattered through the nuclear cavity. In doubly-stained material the accessory chromosomes appear as two red rods lying side by side among the violet V-shaped chromosomes. The latter now split from apex to base, opening out to form double V's and, sometimes after, sometimes during this process are drawn into the equator of the first maturation spindle (Pl. I., Figs. 16–20). In the metaphase the accessory chromosomes always take a peripheral position, are connected by linin fibers to one pole only, and are drawn to this pole before the daughter V-shaped chromosomes are more than half way to their destination (Pl. I., Figs. 23, 24).

According to Wagner — "bei der ersten spermatocytentheilung theilt sich der Nucleolus entweder in der Ebene der Äquatorialplatte mit den Chromosomen zusammen oder auszerhalb denselben neben einem der spindelpole." In the latter case he believes it to be cast out into the cytoplasm. Here, as elsewhere, what he considers to be a "Nucleolus" is without doubt the pair of accessory chromosomes which often lie closely apposed to one another and arrive at one pole before the other chromosomes. Their eccentricity of position might mislead one into thinking that they are being thrown out of the nucleus.

In every case where a section is found cutting through the equatorial plate of the primary spermatocytic monaster transversely, cross-sections are found of the nineteen double V-shaped chromosomes and frequently are found also the accessory chromosomes which appear larger because of their oblique position in relation to the spindle axis. Their nearness to the cell-wall is here quite clearly demonstrated (Pl. I., Figs. 21, 22). During the telophase the nineteen V-shaped chromosomes enter into a partial rest, becoming granular but not forming a reticulum, while the accessory chromosomes in one of every two daughter nuclei stand out in a striking manner as two densely stained rods against the granular background and in sections stained with Hermann's method, their affinity for the safranin at a time when the other chromosomes take the gentian violet, makes them still more prominent (Pl. I., Fig. 25).

The nuclear membrane now forms, one nucleus containing a pair of accessory chromosomes, the other none (Pl. I., Fig. 26), and the V-shaped daughter chromosomes, attached by their apices are quickly drawn into the equator of the spindle for the second maturation division (Pl. I., Fig. 27). Frequently the cell body of the primary spermatocyte does not undergo division until toward the close of the telophase of the second maturation division.

SEQUENCE OF REDUCTION AND EQUATIONAL DIVISIONS.

Most authors now grant that one transverse and one longitudinal division obtain in the maturation divisions of the germ cells, but there is still a lack of argreement in regard to the sequence of these two divisions. There has been a decided majority in favor of the view that the longitudinal division occurs first. Montgomery has emphasized the importance of determining the origin of the chromosomes in order to get at the truth of this matter and he claims to have established the fact that "the heterotypic mitosis, the first maturation mitosis, is not an equation division but separates entire univalent chromosomes, while the second maturation mitosis is equational." This conclusion is supported by the work of Korschelt on the ovogenesis of an annelid, by the work of Henking and Paulmier on Hemiptera, fo Miss Nichols on isopods and by several others. There is the possibility that uniformity in regard to the sequence of the divisions does not exist since in either case the ultimate result is the same, but if there is uniformity the spider spermatogenesis seems to me an especially good field for determining this point and it shows strong grounds for believing that the first division is a reduction division. The process is as follows: When pseudo-reduction occurs in synapsis, giving rise to nineteen loops of different lengths, there is at first no split visible in the thread of the loop. When the split does appear it becomes steadily more noticeable during the growth period and is then obscured in the condensation of the chromatin to form the thickened V-shaped chromosomes (text figure, *a* and *b*). In the prophase of the primary spermatocyte, the V's split from apex to base, parting along the line, I maintain, of the original, longitudinal split of

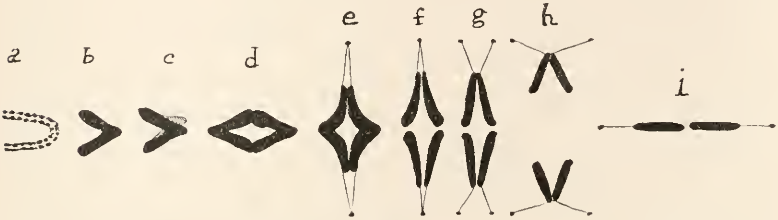

FIG. 1

the loops in the spireme (*a* and *c*), and then open out into double V's (*d*). In the metaphase these double V's are so placed at the equator of the spindle that the longitudidal split is parallel to the spindle axis. This can be determined with certainty since often the splitting occurs after the chromosomes have taken up their position in the equatorial plane and can be seen at different stages of the process, the line of the split being always parallel to the spindle axis (Pl. I., Figs. 18, 19, and text figure, *e*).

Double spindle fibers connect the ends of the double V-shaped chromosomes with the centrosomes. To express it in another way, — after a V-shaped chromosome has become a double V by a split passing longitudinally along each arm, each half of each arm is connected by one linin fiber with the centrosome, but since the distal ends of every such pair are in close juxtaposition, we have the appearance of double spindle fibers passing from them to the poles. The first maturation division then takes place through what corresponds to the apex of the original V's and is a transverse or reduction division (*f*). When the centrosomes divide to form the daughter centrosomes for the second maturation spindle, one of each pair of linin fibers remains attached to one of the daughter centrosomes. When the latter have moved in opposite directions and have taken up their positions at right angles to the position of the mother centrosome (thus becoming the poles of the second maturation spindle) the V-shaped daughter chromosomes, formed from the longitudinal splitting of a single arm, are left attached by their apices in the equator of the spindle. The space between the arms of these single V's corresponds to the space between the halves of the longitudinally split spireme and, therefore, when the arms of the V are drawn to opposite poles, we plainly have an equational division (*g*, *h*, *i*).

To sum up: The first division occurs at a point corresponding to the bend of the loop in the spireme while the second occurs along the line of the original, longitudinal split of the spireme. Such an interpretation must surely be the true one if it be granted that the loops arise in the synapsis by a conjugation, end to end, of like chromosomes. And, even if the loops are supposed to originate by the spireme simply breaking up into the reduced number of chromosomes, still the sequence of the divisions would be as given above.

SIGNIFICANCE OF THE ACCESSORY CHROMOSOME.

Considerable attention has been paid to the distribution of accessory chromosomes to the spermatids because opinions have differed in regard to it. Nothing could be clearer than the fact that, in the spider, during the first maturation mitosis, the two accessory chromosomes pass bodily over into one of the two daughter cells. In the second maturation mitosis the question is far more difficult to answer, partly because but one half of the cells contain the accessory chromosomes anyhow and partly because the V-shaped chromosomes attached to the equator of the spindle by their apices, cause a branching in the equatorial plate and thus the monaster is a much less clear subject for study than is found in the first spermatocytic monaster. A number of favorable cases were found, however, in which the two accessory chromosomes pass to but one pole and such cases, together with a careful study of the spermatids, have convinced me that not only in the first division, but also in the second the accessory chromosomes pass to but one pole, and are therefore, distributed to only *one fourth* of the spermatids (Pl. I., Fig. 28). In this respect my results differ from all other workers yet heard from. Henking and Paulmier found the hetero-chromosomes dividing in the first spermatocytic division, but passing undivided to one daughter cell in the second; McClung, Sutton and Blackman hold just the reverse. They find them taking no part in the first but dividing in the second division. In either of the above cases the result would be the same, *i. e.*, one half of the spermatids would contain no heterochromosomes, while the other half would contain them and could be called what Henking has styled "bevorzugten Tochterzellen." Montgomery finds the heterochromosomes dividing like the ordinary chromosomes in both divisions and they are, therefore, present in all of the spermatids, while in the spider they seem to stand aloof in both of the maturation mitoses and are, therefore, distributed to but one fourth of the spermatids.

In consideration of our limited knowledge in regard to the heterochromosome, and of the fact that nothing is known as to what part this peculiar nuclear element plays in fertilization, it is perhaps premature to say anything in regard to its significance.

McClung has suggested that the heterochromosomes might be sex-determinants and he is supported in this view by Sutton, Blackman and others. Paulmier regarded them as degenerating chromatin. Montgomery agreed with him in this and considers that they are "chromosomes that are in the process of disappearance, in the evolution of a higher to a lower chromosomal number." If only one fourth of the spermatids contain the heterochromosomes, as in the spider, they can scarcely be sex-determinants. Neither do they here show the characteristics of degenerating chromatin. That they represent some form of specialized chromatin, I cannot doubt, but that they are specialized for some metabolic function and comparable to nucleoli, as has been suggested by Montgomery, does not seem to me probable in view of the facts. I, therefore, venture to offer a fourth theory in regard to the function of the accessory chromosomes in the spider.

The breeding season of *Agalena nævia* comes late in the summer, the eggs being laid in August and September. While examining preparations made late in September I noticed a great many degenerating cells side by side with the ripe spermatozoa found in abundance in the lumen of the testis and in the ducts (Pl. II., Fig. 49). These cells are without nuclei and many of them are fragmented. My first conclusion was that the presence of so many fragmenting cells was due to the lateness of the season but on examination of sections prepared early in August a few cells undergoing degenerative processes were found even though but few spermatozoa had yet reached maturity. Sections made from the testis of *Pholcus phalangiodes* at the very height of the breeding season, in July, were most convincing. In *Pholcus* the spermatozoa are much more elongated than in any other species of spider examined (Pl. II., Fig. 51), but when mature they curl up as the others do. In the lumen and ducts were found hundreds of spermatozoa thus coiled up but a far greater number of round cells with rounded nuclei in which the linin and chromatin are no longer distinguishable. These cells vary in size — some as large as the germ cells, some very minute and in every way they resemble cells in the process of degeneration (Pl. II., Fig. 50, *d*). Further study showed that as a rule the degeneration does not begin until after the spermatozoa have been emptied from the cysts into the lumen. The process then goes on very rapidly. Probably the degenerating cells of *Pholcus*, if examined near the close of the breeding season would be found to have lost their nuclei like those of *Agalena nævia*. It was not found possible to make an accurate count of the two kinds of cells in the ducts and hence it was not possible to determine with certainty their relative numbers but it was estimated that there were about three times as many of the degenerating cells as of the normal, ripe spermatozoa. What could be the determining cause? It will be remembered that there are three times as many spermatozoa without the accessory chromosomes as with them, and it occured to me that possibly only one fourth of the spermatids, the "bevorzugten zellen," become functional spermatozoa. Platner and O. Hertwig have shown that the two divisions which form the polar bodies of the egg are homologous to the last two divisions of male germ cells but that while in the female germ cell the products of division are very unequal in size and only one of the four is functional, in the male germ cell they are equal in size and each of the four is functional. If it can be proven that three fourths of the sperm cells are aborted, just as three fourths of the ova are, the parallelism in the spermatogenesis and ovogenesis will be found to be even more complete than has hitherto been supposed. There are at least *a priori* reasons for believing such to be the case and I was still further strengthened in this position when my attention was called to a short paper by Meves in which he describes the finding of "polar bodies" in the testis of the honey bee and other Hymenoptera. "Die Spermatocyten 1. Ordnung bei den genannten Hymenopteren stoszen ebenso wie sonst die Eier bezw. Ovocyten 1. Ordnung nach einander zwei "Richtungskörper" aus; von diesen besitzt jedoch nur der zweite einen Kern, während der erste ausschlieszlich von Cytoplasma gebildet wird . . . . Die ersten Richtungskörpen gehen nach einiger zeit zu Grunde. Die zweiten Richtungskörper dagegen beginnen ebenfalls sich zu Sprermien zu entwickeln, wobei ihre Kerne dieselben Veränderungen wie die Kerne der groszen zellen und zeitlich parallel mit ihnen durmachen-jedoch scheint dieser Entwickelungsprozesz schlieszlich, wenn auch erst sehr spät, zum Stillstand zu kommen und in Degeneration überzugehen."

We might here mention once more that the accessory chromosomes show a staining reaction throughout which the other chromosomes show at the height of mitosis and which some think to be due to the presence of a larger amount of nucleinic acid at that time. Now while staining reactions are not a safe criterion, they may give us at least a rough test of the chemical conditions in a cell, and since the nuclei of spermatozoa have been found to contain a maximum of nucleinic acid, and since the "bevorzugten zellen" in the spider might be richer in nucleinic acid, on account of the presence of the accessory chromosomes, than the remaining three fourths of the male germ cells which are not so favored, we might here see a new significance in the accessory chromosomes.

Spermatids and Spermatozoa.

A study of the spermatids will throw further light upon the subject of the distribution of the accessory chromosomes. In most cases it is evident that when the secondary spermatocytes, which do contain the accessory chromosomes, divide, the latter pass to but one of the resultant spermatids (Plate II., Figs. 29–33). Figs. 34 and 35 show spermatocytes which are not "favored cells." Sometimes each of the two spermatids resulting from the division of a favored spermatocyte seems to contain one accessory chromosome, but I think this should be regarded as an exceptional case (Pl. II., Fig. 37). At a later stage in the formation of the spermatozoön it is impossible to determine which cells contain the accessory chromosomes and which do not. At the beginning of the process of the condensation of the chromatin to form the head, the two accessory chromosomes become closely applied to one another, then appear to fuse together. They still take the safranin stain and form a center into which the chromatin reticulum which takes the violet stain, is gradually drawn until all of the chromatin forms a compact mass stained brilliant red. In the spermatids which lack the accessory chromosomes the chromatin at one point in the reticulum forms a net-knot which now takes the safranin stain and it becomes the center of condensation and so simulates the appearance of the favored cells. At such a stage one might be mislead into thinking that every spermatid contains the accessory chromosomes. This stage in *Agalena* is shown in Fig. 38 and in *Epeira*, Figs. 42–44. In all cases the chromatin assumes a crescentic form, the anterior end bends in, the posterior end folds over it and we see the mature spermatozoa each consisting of a crescent-shaped nucleus, covered by a pellicle of cytoplasm and curled up. In this condition they are found in abundance in the lumen of the testis, in the ducts and in the pedipalps (Figs. 39–41).

In Fig. 48 is pictured a spider spermatozoön after Wagner in which he demonstrates a tail. This is of special interest to me because I have not succeeded in finding an organ of locomotion in connection with the spermatozoön. As he also worked upon *Agalena*, I am surprised to find my results so different from his. He claims that "Die Spermatozoen haben auf gewissen Stadien bei allen Species einen typischen Schwanz mit Achsenfaden. Der Achsenfaden bildet sich im Protoplasma der Spermatocyte (resp. Spermatide) zuerst als ein kurzes Stäbchen welchem bisweilen einige Archoplasmakörnchen anliegen. Mit dem Kerne verbindet er sich erst nach dessen Umwandlung in die Chromatinplatte."

"Wo sich Achsenfaden und Chromatinplatte verbinden liegt am Rande der letzeren ein Zähnchen." In striving to get light upon these points I have studied the spermatozoa of about a dozen different species of spiders, have stained them *intra vitam*, have made smear preparations fixed by heating at the boiling point, have studied, with painstaking care, sections fixed and stained in a great variety of ways, and in no case has a tailed spermatozoön been found with the exception of *Pholcus* and even here the elongated portion which might be looked upon as a tail, stains more like a middle piece and its cytoplasmic origin is questionable (Fig. 51). My belief is that the "tail" which Wagner saw was nothing more nor less than the outline of a vesicle which is nearly always in evidence when the spermatid is being transformed into the spermatozoön, and that what he describes as a little tooth is really nothing more than the anterior end of the head bent under, although in this case there should be a rounded bend instead of what he figures (Figs. 46, 47). I would be glad to believe in the existence of a tail and have earnestly tried to demonstrate it but so far the method of locomotion of the spider spermatozoön remains an unsolved mystery to me. In a future paper I hope to be able to give some light upon this subject as well as upon the rôle played by the accessory chromosomes in fertilization. As my work now leaves it, the functional spermatozoa would contain an uneven number of chromosomes — nineteen ordinary ones and two accessory chromosomes, and it is not clear how the somatic number of forty chromosomes is made up in the cleavage nucleus of the ovum unless it be found that accessory chromosomes are thrown off in the polar bodies, thus leaving the mature ovum with only nineteen chromosomes. These nineteen, added to the twenty-one brought in by the spermatozoön would make the total required; forty chromosomes.

SUMMARY.

1. The spermatogonia contain two accessory chromosomes and thirty-eight other chromosomes.

2. In the primary spermatocytic division the two accessory chromosomes pass over undivided into one of the daughter cells. The reduced number of other chromosomes is nineteen and these divide transversely.

3. In the secondary spermatocytic division, the two accessory chromosomes again pass over undivided into one of the daughter cells. The nineteen other chromosomes divide longitudinally.

4. Only one fourth of the spermatozoa contain the accessory chromosomes.

5. Apparently the remaining three fourths of the spermatozoa degenerate after almost or altogether reaching maturity. In this respect they are regarded as homologous to the polar bodies thrown off by the ovum.

THE UNIVERSITY OF PENNSYLVANIA,
June, 1904.

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Explanation of Figures.

All drawings were made at a magnification of about 2,000 diameters and then reduced one third. All figures were drawn from Agalena unless otherwise specified, and were drawn with the aid of the camera lucida.

Plate IX.

Fig. 1. Spermatogonium in resting stage.

Fig. 2. Pole view of equatorial plate of spermatogonic monaster.

Fig. 3. Spermatogonic monaster.

Figs. 4–5. Anaphase of spermatogonic monaster.

Fig. 6. Daughter cell of last spermatogonial division showing disintegration of the chromosomes.

Figs. 7–8. Resting cells.

Fig. 9. Formation of spireme, "synapsis."

Fig. 10. Completion of spireme.

Figs. 11–13. Coarse spireme with longitudinal split.

Figs. 14–15. Shortening and thickening of loops of segmented spireme.

Fig. 16. Prophase of primary spermatocyte with loops transformed into V-shaped chromosomes.

Fig. 17. Splitting of chromosomes.

Figs. 18–20. Primary spermatocytic monaster.

Figs. 21–22. Sections of equatorial plate.

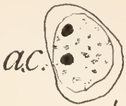
Fig. 23. Primary spermatocytic metaphase.

Fig. 24. Primary spermatocytic anaphase.

Fig. 25. Late anaphase or telophase.

. 26 . Reconstruction of daughter nuclei.

Figs. 27–28. Secondary spermatocytic monaster.



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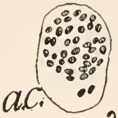

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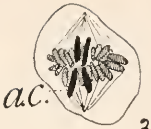

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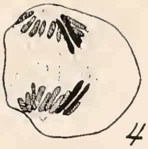

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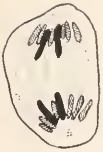

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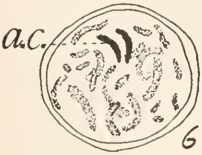

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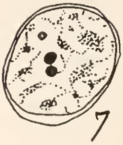

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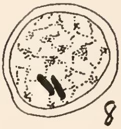

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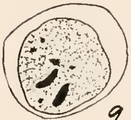

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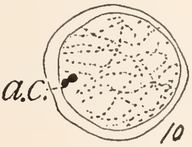

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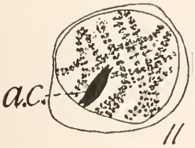

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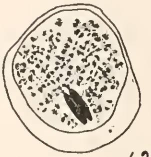

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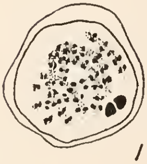

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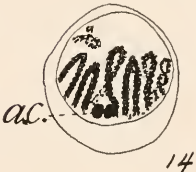

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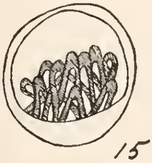

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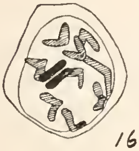

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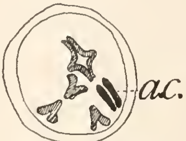

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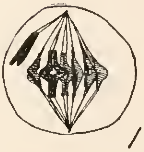

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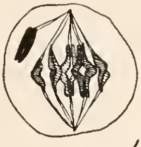

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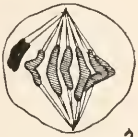

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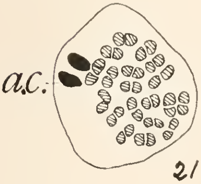

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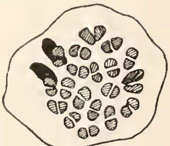

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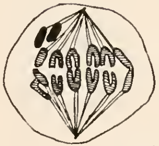

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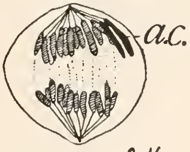

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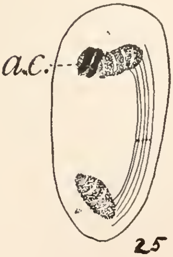

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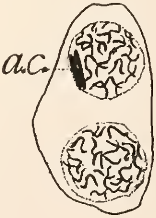

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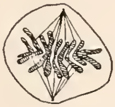

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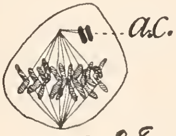


28

PLATE X.

FIGS. 29–39. Spermatids.

FIGS. 40, 41, 46, 47. Spermatozoa.

FIGS. 42–45. Spermatids of *Epeira sclopetaria*.

FIG. 48. Spermatozoön of *Agalena*, nach Wagner.

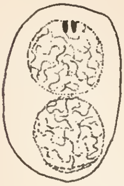
FIG. 49. Normal spermatozoa and degenerating cells. *Agalena*.

FIG. 50. Normal spermatozoa and degenerating cells. *Pholcus*.

FIG. 51. Spermatozoa of *Pholcus phalangiodes*.

ABBREVIATIONS.

a.c., accessory chromosomes ; *sp.*, spermatozoön ; *d.c.*, degenerating cells ; *t.* tail of spermatozoön, nach Wagner ; *v*, vesicle.



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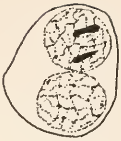

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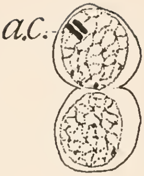

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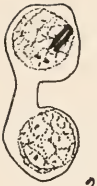

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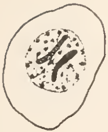

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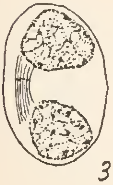

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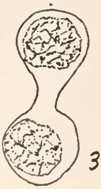

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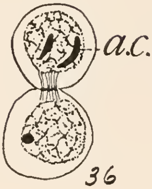

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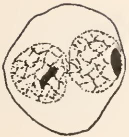

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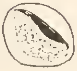

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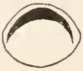

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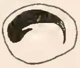

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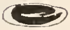

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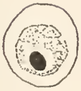

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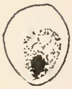

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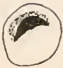

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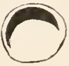

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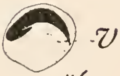

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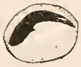

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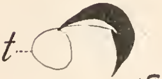

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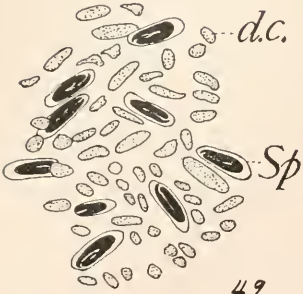

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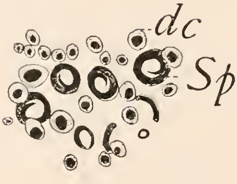

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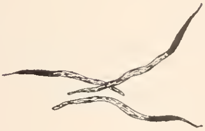


51

THE FORMATION OF THE INTERIOR CELLS IN THE SEGMENTATION OF THE FROG'S EGG.

MARGARET REED.

Sections through the egg of the frog in the early stages of segmentation show that the cleavage planes extend from the surface inwards forming but one layer of cells. Later cleavage stages, however, show certain cells entirely within the interior of the egg, so that the cells of the dark pole appear to be arranged in two layers about the segmentation cavity.

Former observers supposed this appearance to be caused by a cleavage plane coming in parallel to the surface of the egg (delamination) probably between the 32- and 64-cell period.

Following Professor Morgan's suggestion, I attempted to determine whether a delamination really occurs or whether the interior cells are produced in some other way.

If the delamination occurs between the 32- and 64-cell stages we should expect to find the interval between these cleavage stages, as seen on the surface, longer than that between any two preceding divisions, because somewhere during this time, the cells would have divided by planes, which do not appear on the surface.

In order to test this point, I made a number of observations on the living egg, noticing the times of the surface divisions. I found no marked difference in the length of the interval between any two divisions, but the cleavage took place in a regular rhythm. There was usually a period of about one hour from the first appearance of segmentation in the cells of the dark pole until the next cleavage again began to appear there. This is shown in the following table.

9:25 A. M.	Beginning of	2-cell	stage	at dark pole.
10:15 "	"	4	"	"
11:15 "	"	8	"	"
12:10 P. M.	"	16	"	"
12:55 "	"	32	"	"

1:50 P. M.	Beginning of	64-cell	stage	at dark pole.
2:40 "	"	128	"	"
9:25 A. M.		8	"	"
9:30 "	"	16	"	"
10:35 "	"	32	"	"
11:35 "	"	64	"	"
12:25 P. M.	"	128	"	"
1:40 "	"	256	"	"

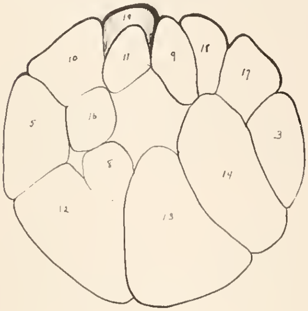
From this we see that if the delamination should occur between the 32- and 64-cell periods the division must take place at this period twice as fast as any other division. This of itself seemed improbable. I therefore preserved series of eggs taken every five minutes during the interval between the 32- and 128-cell divisions and later sectioned them in order to determine how the interior cells arise.

The eggs were cut about 20 μ thick and all the sections drawn with the aid of a camera lucida. Each cell of the egg was numbered and traced through the series of sections, in order to determine not only how many cells were present but also to ascertain how many came to the surface and how many were entirely inside. Two series taken every five minutes between the 32- and 128-cell stages were counted with the following as some of the results:

Supposed Number of Cells.	Actual Number of Cells.	Cells Inside.	Superficial Cells.
32	28	0	28
32	32	0	32
32–64	37	3	34
32–64	45	5	40
32–64	53	6	47
64	64	15	49
64–128	105	26	79

If delamination took place between the 32- and the 64-cell period, sections of an egg at the 64-cell stage should show 64 cells coming to the surface, plus a certain number of cells inside, but this, as the table shows, is not the case.

Sections through the egg as late as the end of the 32-cell stage show that all the cells are divided by cleavage-planes, which appear on the surface. Sections through the eggs of the series between the 32- and 64-cell stage show an increase in the number of superficial cells[1] as well as of those which are wholly within the egg. At the end of the 64-cell stage there are 64 cells present in the egg, a number, about 12, of which do not appear on the surface. Also the series between the 64- and 128-cell cleavage stages show an increase in the number of cells coming to the surface, as well as the number of cells of the interior, so that there is no cleavage stage between which and the following stage delamination takes place.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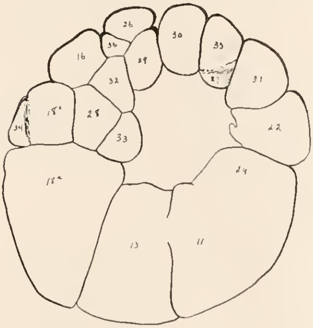
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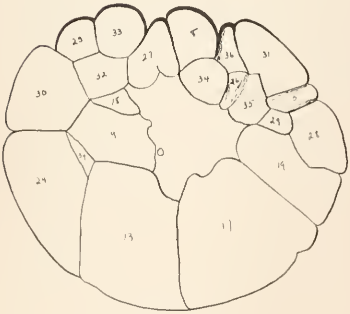
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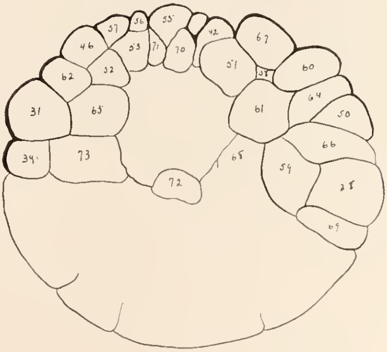
III.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IV.

Figs. 1, 2, 3 and 4 show sections through the middle of eggs of 28, 53, 64 and 105 cells respectively. A number of cells in these figures have the appearance of being pushed or crowded into the interior, that is, the portion of the cell which comes to the surface is quite small compared with the size of the cell which lies mainly within the egg.

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[1] In the cleavage of the frog's egg, the division for the next cleavage stage begins in the cells of the dark pole, while the cells of the light pole are completing the division for the preceeding cleavage stage.

This appearance of the cells seems to be caused by the division planes coming in more or less obliquely so as to cut off one cell with a small surface area as No. 9, Fig. 1. At the next cleavage, the division of this cell would give rise to one cell entirely within the egg and one coming to the surface. Cell 27, Fig. 3, shows the beginning of such a division while cell 70 or 71, Fig. 10, may have been formed in this manner. The following division of such a cell would then form cells lying entirely within the egg.

So far as I have been able to trace, the yolk cells also show no cutting off of interior cells during the early cleavage stages. Such a cell as 72 in Fig. 4, is but the end of a cell which other sections show coming to the surface.

These observations show that there is no delamination division cutting off a number of cells parallel to the surface but that the first formed cells of the interior are produced by the same division planes as are the cells of the surface and by their subsequent division form the cells of the interior.

Columbia University,
December 13, 1904.

ON THE CONDITIONS DETERMINING THE DISPOSITION OF THE CHROMATIC FILAMENTS AND CHROMOSOMES IN MITOSIS.

RALPH S. LILLIE.
RESEARCH ASSISTANT OF THE CARNEGIE INSTITUTION.

Regarded from the purely physico-chemical or physiological standpoint protoplasm is essentially a complex aggregate of water and various colloidal and crystalloidal substances of which electrolytes form a chief part. It is also important to note that the *distribution* of the substances composing this aggregate is often, perhaps always, of a highly definite and specific kind; in recognition of this fact a more or less clearly defined "structure" has always been ascribed to protoplasm. Strictly speaking the term "structure" must include in its significance the distribution, grouping and relative positions of *all* the different cell-constituents; ordinarily, however, we do not regard the water and the more readily diffusible substances as distinctively structural elements; in general we mean by such elements those whose position in the complexus remains relatively constant and which give the form to the whole. On this definition we observe that it is primarily the *colloidal* substances of the cell that determine by their arrangement, distribution, and state of aggregation the particular type of structure that is presented; in other words, colloids form the basis of protoplasmic structure.

The reason for this is to be sought in a consideration of the general physico-chemical characteristics of this class of substances. The colloids of protoplasm, by virtue of their slow diffusibility, inability to penetrate one another, slow penetrability or virtual impenetrability to many crystalloids,[1] and relative unliability to chemical change, form throughout the cell a substratum which admits of the persistence within the cell-limits of a high degree of non-homogeneity. This condition is evidently indispensable for the existence of the localized differentiations that constitute organized structure. Its physiological counterpart is seen in the fact that there are constantly taking place within the single cell a multiplicity of diverse and often contrary, yet simultaneous processes; these normally are so coördinated that the entire system is enabled to maintain itself in equilibrium and to carry on its vital functions within a certain more or less limited range of conditions. Thus the possibility of this coördinated differentiation of structure and function, which is perhaps the most distinctive peculiarity of living matter, appears to depend on the above properties of the colloids and on the special manner in which these substances are disposed within the cell.[1]

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[1] Seen in the non-permeability of colloidal membranes to many dissolved substances.

It is, therefore, to the conditions determining the disposition and state of aggregation of the colloids within the cell that we must look, when we attempt to explain the mode of origin of any given one of its structural features. Perhaps the most constant and distinctive of these features is the division into nucleus and cytoplasm. The proteids, which are the chief colloids of these two regions of the cell, are widely different in chemical nature; this implies a difference in their electrical properties, and with this is probably correlated the very typical segregation which they exhibit — the nuclear proteids forming a central aggregate which is almost always separated from the cytoplasmic proteids by a distinct membrane apparently impermeable to both. This seems the essential characteristic of the nucleated cell. A further peculiarity of the nuclear colloids is the remarkably definite disposition which they show at times; this is especially marked at the period of cell-division, when they form arrangements and go through movements of so characteristic a kind that the name *karyokinesis* has been given to the entire process. It is this peculiar mode of distribution of the nuclear colloids that I propose briefly to consider in the present paper: the movements and grouping of the nuclear colloidal aggregates, chromatic filaments and chromosomes, though apparently complex, are,

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[1] For two important recent discussions of the part which colloids play in protoplasm, cf.: Hofmeister, "Die chemische Organisation der Zelle," Braunschweig, Vieweg, 1901; and Pauli, "Der kolloidale Zustand und die Vorgänge in der lebendigen Substanz," Braunschweig, Vieweg, 1902.

I believe, due essentially to a series of relatively simple conditions the nature of which I shall attempt to analyze below.

What then are the conditions that determine the disposition adopted by the chromosomes and the chromatic filament in the dividing cell? In the prophase of mitosis the chromatin[1] becomes disposed in so many cells in the form of one or more elongated deeply staining[2] filaments coiled within the nuclear membrane into a roughly spiral form, that the arrangement is regarded as typical. This is the well-known "spireme" stage. The spireme then segments into the definite number of chromosomes characteristic of the cell; the nuclear membrane disappears and the chromosomes are drawn into the equatorial plane of the cell; here they assume the arrangement known as the "equatorial plate" in which, it is to be noted, a distinct interval separates each chromosome from its neighbors. Each then divides, typically by a process of longitudinal splitting, into the two daughter-chromosomes which recede toward opposite poles of the cell; here later the daughter-nuclei are reconstituted from the two groups of daughter-chromosomes thus formed.

Both the adoption of this remarkable spiral arrangement and the manner in which the chromosomes become disposed in the equatorial plate are, I shall attempt to show, dependent mainly upon one simple physical property which the particles of chromatin possess in common with other similarly charged colloidal particles — namely, the property of mutual repulsion. It may now be considered as finally established that a colloidal substance in solution is in the condition of a more or less finely divided suspension, each particle of which is at a different electrical potential from the adjoining layer of liquid, *i. e.*, carries an electrical charge. The sign of the charge is positive in the case of basic, negative in the case of acid bodies, since the former liberate negative (OH^-) ions, the latter positive (H^+) ions ; the colloid particles themselves are thus left with free positive or negative charges respectively. All particles in a given colloidal solution, having like charges, must for this reason repel one another. Apparently the stability of a colloidal system depends upon this mutual repulsion of the particles of solute, since neutralization of the charge, as by the action of ions of opposite sign, results in an aggregation of the particles and the removal of the colloid from solution ; to this action is to be ascribed the precipitating effect of ions.

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[1] This is the cytological term; chemically this substance is undoubtedly nucleoproteid in nature; the chromosomes are probably similar to sperm-nuclei in their composition, as we may infer from the similarity between the micro-chemical reactions of the two. The nuclein of sperm-heads is relatively simple in composition and contains a large proportion of nucleic acid. For a general account see R. Burian in Asher u. Spiro's Ergebnisse der Physiologie, 3, 1894, I. Abtheilung, *Biochemie*, pp. 48–106.

[2] The increased depth of stain is an indication of increased acidity, *i. e.*, increased liberation of hydrogen ions; this involves an increase in the electrical negativity of the colloidal substance and consequently in the force with which adjacent portions of the filament repel one another.

If the particles composing the chromatic filaments and the chromosomes similarly repel one another — as in accordance with their colloid nature they must do — mutual repulsion will play an important part in determining the disposition which these structures adopt within the cell. The chromatin being an acid body must liberate hydrogen ions and become negatively charged, and this to a greater degree the higher its proportion of nucleic acid. I have elsewhere pointed out that sperm-nuclei, which are rich in nucleic acid, show a particularly strong tendency to travel with the negative stream when an electric current is passed through a solution containing them ; this fact may be regarded as confirmatory of the view that the chromosomes, which in composition are probably very similar to sperm-nuclei, are likewise negatively charged bodies.[1]

Again the prevailingly central position of the nucleus within the cell — especially the cell about to undergo mitosis — indicates that there exists within the cell an influence tending to draw the nuclear colloids toward a central position. There are reasons for regarding this influence also as electrical in its nature ; it seems highly probable that in the dividing cell the intra-nuclear colloids and the cytoplasmic colloids are opposite in their electrical properties[2] ; they are certainly contrasted in their general chemical behavior; at all events they react toward acid and basic staining reagents in precisely opposite manners. This would indicate that the prevalent cytoplasmic colloids are preponderatingly[1] basic in character, *i. e.*, that their particles liberate hydroxyl ions chiefly, and are accordingly for the most part positively charged. It is conceivable that the hydrogen ions from the nuclear proteids and the hydroxyl ions from the cytoplasmic proteids, both of which ions have high migration velocities, find their way throughout the entire cell and unite with each other to form water. The colloids themselves, on the other hand, though of opposite sign, are unable so to unite, or can unite only partially, on account of the impossibility of their diffusing through one another; they are therefore left in the cell with free and opposite charges. Possibly a partial union of the nuclear and the cytoplasmic colloids may take place at the boundary between the two aggregates; it is known that colloids of opposite electrical sign will precipitate each other when their solutions are mixed, presumably by each effecting a neutralization of the other's charge.[2] Now it is a very remarkable fact that the nucleo-proteids of the cell are almost invariably separated from the cytoplasmic proteids by a well defined membrane, the "nuclear membrane," which is very possibly a precipitation-membrane formed at the surface of contact between the two oppositely charged colloidal aggregates. If this is so, it becomes intelligible why further neutralization of the charges is prevented and how each set of colloids is enabled to retain its charge in the presence of the other. The condition thus reached is that of a negatively charged aggregate, *viz:* the nucleus with its contained chromatin, in the midst of a positively charged field consisting of the numerous positively charged colloidal particles of the cytoplasm.[3]

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[1] R. S. Lillie, *American Journal of Physiology*, 8, 1903, p. 273. Also Biol. Bull., 4, 1903, p. 175.

[2] Proteids are amphoteric bodies, *i. e.*, may liberate a preponderance either of hydrogen or of hydroxyl ions according to conditions.

The central position of the chromatin is now easily explained as due to the attraction which the aggregate of oppositely charged cytoplasmic colloids exercise upon it, the resultant effect of all these attractive forces being to draw the chromatin toward the center of the cell. On the other hand adjacent chromosomes or adjacent portions of the chromatic filament are by their own mutually repellent action exposed to an opposite influence which resists their approach toward one another. Hence the position of equilibrium, *i. e.*, the stationary position which they finally adopt, must be one where these two opposed sets of influences exactly balance each other.

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[1] Cf. R. S. Lillie, *loc. cit.*

[2] Biltz, *Berichte der deutschen chemischen Gesellschaft*, 37, 1904, p. 1095.

[3] This suggests J. J. Thomson's conception of the atom as a system of negative electrons moving in a field of uniform positive electrification. It would be strange if such a parallel should exist between the respective units of living and of lifeless matter.

The formation of the spireme and of the equatorial plate may then be ascribed to these two chief conditions, one the attractive influence that draws the chromatin toward a central position and tends to keep it there, the other the mutually repellent action of the chromatin particles themselves. The stationary position is one of equilibrium between these two opposed tendencies.

I should add here, in reference to the striking fact that the chromosomes at the metaphase occupy positions side by side *in a single plane* — that of the equator of the cell — that this remarkable peculiarity of disposition also remains to be accounted for. As yet, however, I know of no experimental facts bearing directly on this problem; and in the following explanation of the manner in which the equatorial plate is formed, I shall assume from the first that the chromosomes are free to move only in this single plane, without as yet attempting to explain why this is so.

Experimental.

If the conditions of spireme-formation and of equatorial plate-formation are similar to those suggested above, it should be possible to simulate these phenomena experimentally by making use of artificial filaments composed of mutually repellent units arranged in rows, and by subjecting these to the action of some centrally attracting force. The disposition and relative positions assumed by such filaments should, if the above hypothesis is correct, resemble those exhibited by the chromatic filament and chromosomes in the dividing cell.

In pursuance of this idea I have experimented with filaments consisting of rows of floating magnetized needles. The experiments of Alfred Mayer on floating magnets are well known to physicists; he studied many years ago the behavior of groups of small magnetized needles which were floated by being passed through small discs of cork and placed on the surface of water in such a manner that all the magnets were vertical in position and similarly oriented, *i. e.*, with all the north (or south) poles uppermost. Such floating needles repel one another with a force inversely proportional to the square of their distance apart. If then over such a group floating with (for example) north poles uppermost a large bar-magnet is suspended in a vertical position with its south pole downward, all the needles are, by the horizontal resultant of the attractive force of the large magnet, drawn in toward a central position immediately below the latter ; here they adopt arrangements of perfectly definite and regular configuration, whose exact form varies with the number of needles but shows great constancy for any given number.[1] The condition of stability of such systems is that the mutual repulsion which the small magnets exercise upon one another shall be exactly balanced by the centripetal attractive force due to the large magnet.

In their manner of grouping such floating magnets exhibit a close resemblance to the chromosomes of many equatorial plates, for example, those of the sea-urchin egg (seen in face). So far as I am aware, however, no attempt has hitherto been made to explain the grouping of chromosomes as due to conditions of the above kind. The following experiments have been designed with a view to testing this hypothesis.

To simulate the nuclear chromatic filament, the following procedure is employed : Small, similarly oriented, magnetized needles are strung at short and regular intervals (say 6 millimetres apart) along a delicate silk thread, preferably a single silk filament ; each needle is passed through a small cubical piece of cork ; the entire filament can then be floated on the surface of water with the needles vertical in position. When left undisturbed such a filament tends to be pulled out into a straight line by the mutual repulsion of its units ; this form would be taken if the filament were ideally flexible and the supporting fluid devoid of viscosity, since then the average distance of the needles apart would be as great as possible under the conditions. Actually, however, the straight form is only approximated.

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[1] Figures showing these arrangements will be found in any good text-book of physics, for example Ganot's.

Such a filament may be caused to assume the spireme form as follows: A large bar magnet is suspended vertically over the filament with its north pole next the projecting south poles of the needles (or *vice versa*) and at a suitable distance from the latter. It is then found that the filament is drawn together by the attraction of the magnet into a more or less regular, close coil or spireme-like form, remarkably like that shown by the nuclear filament in the prophase of mitosis. Briefly, the explanation of this behavior is as follows: The filament is, by the attraction of the large magnet, confined within a limited space just as the chromatic filament in the cell is confined by the nuclear membrane. Now, since mutual repulsion tends to prevent approximation of adjacent portions of the filament, the latter is forced so to dispose itself that, while occupying the greatest space possible under the conditions, the average distance between its adjacent portions is also as great as possible. This however leads inevitably to the production of the characteristic coiled or roughly spiral form which is the only one that satisfies these conditions, that is, in which the entire system is in equilibrium.

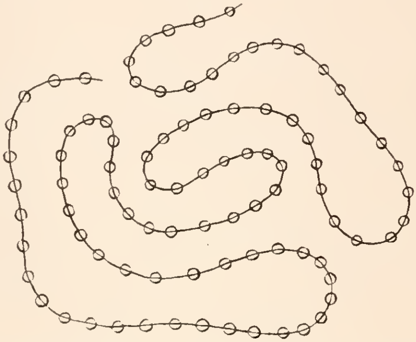

FIG. 1. Artificial spireme from a single filament; the small circles indicate the exact number and relative positions of the magnets.

Figs. 1 and 2 are reproductions of exact drawings showing the form of the artificial spireme in each of two experiments, one with a single filament, the other with six separate filaments.

Their resemblance to the nuclear spiremes is evident. Such spireme figures may exhibit a great variety of forms; at present, however, I shall not attempt to enumerate these in detail ; a later paper will contain drawings showing the exact form assumed by the filaments under a variety of conditions : varying their number, size and structure, as well as the intensity of the central attractive force.

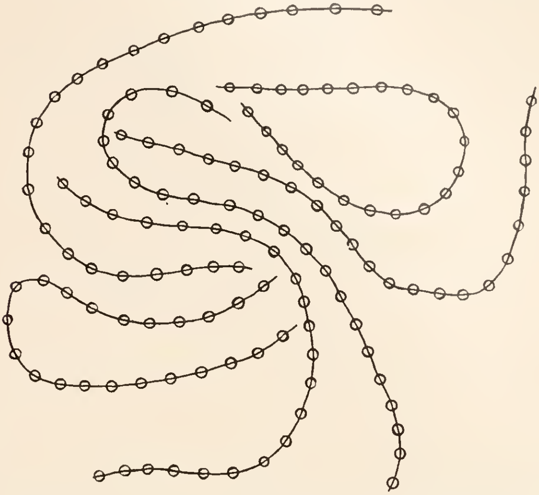

FIG. 2. Spireme from six separate filaments and disposition of individual magnets shown by the small circles.

The formation of the equatorial plate may be simulated by the use of similar floating aggregates of needles, which in this case are strung along flexible wires capable of being bent into any desired shape. In this way the aggregate may be given the

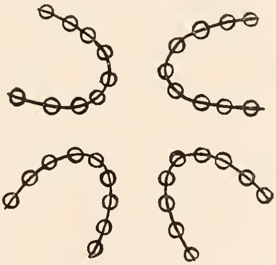

FIG. 3. Arrangement shown by group of four chromosome models each with 9 magnets.

form of any one of the various types of chromosome, and it thus becomes possible to study the effect which changing the form of the chromosomes has upon the configuration of the equatorial plate. These chromosome models repel one another, and, like the isolated needles, they are found to exhibit very constant and regular configurations when subjected, as above described, to the attractive influence of the large magnet. The resemblance to the configurations observed in the equatorial plates of actual cells is often very striking. I have made experiments with varying numbers of such chromosome-like aggregates and have

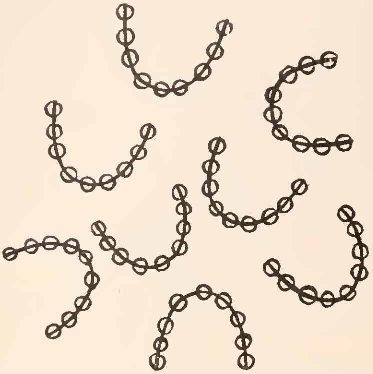

FIG. 4. Arrangement shown by eight loop-shaped chromosome models.

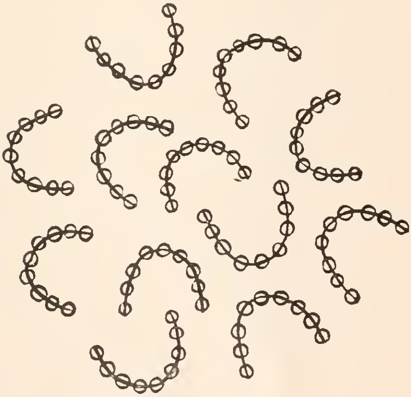

FIG. 5. Group of twelve loop-shaped chromosome models.

attempted to determine what the possible and stable configurations—that is, the conditions of equilibrium—are for any given number of chromosomes up to twelve.

Figures 3, 4 and 5 give exact representations of configurations shown respectively by groups of four, eight and twelve simple loop-shaped chromosome models. It should be added that the number of possible configurations becomes very great when the number of chromosomes is large. In the full paper drawings of a number of these will be given. I shall then consider the condition of their formation in greater detail.

It is my intention to investigate further the possibilities of this type of magnetic model. It is possible that such phenomena as the aggregation of the chromosomes in a single plane, their longitudinal splitting, and the separation of the daughter-chromosomes, may be simulated by means of such models. Much light may thus be thrown upon the mechanics of these characteristic phenomena.

It is to be noted that in experiments of the above type the filaments and chromosomes are arbitrarily confined in their movements to a single plane, that oı the surface of the water. In this respect such models are incomplete, since, in the cell, movement is possible in all three dimensions. It seems clear, however, that, setting aside this partial and incidental limitation, these imitations of cell-phenomena are often surprisingly true to nature. *In so far as regards the mode of disposition* of the chromatic filaments and of the rows of magnets, the observed resemblance may be held to imply an identity in the essential determining conditions. These conditions are those of mutual attractions and repulsions, which are common to both systems. In other respects, it need scarcely be said, the two systems are widely different. Still, so long as science proceeds, as it has always done, by the progressive identification of apparently widely different things it will be necessary to isolate by analysis the features in which otherwise diverse phenomena agree, and to disregard, for the time being, their differences. We may with justification infer from the above that attractions and repulsions—here undoubtedly electrostatic in nature, since colloid bodies are concerned—play a most important part in determining the disposition of certain of the structural elements of the cell. How far such conditions have to do with form-determination in general is a further question, and one that stands in urgent need of thorough-going investigation. It may prove necessary to add electrostatic attractions and repulsions to the list of the conditions determinative of organic form and structure.

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BIOLOGICAL BULLETIN

ORGAN-FORMING SUBSTANCES IN THE EGGS OF ASCIDIANS.

EDWIN G CONKLIN.

With 24 Photomicrographs of Living Eggs of Cynthia (Styela) partita Stimpson.

BY KATHARINE FOOT AND E. C. STROBELL.

That the egg of any animal is composed of "simple, undifferentiated protoplasm" is an article of traditional belief with a large number of zoölogists, and that the cleavage of the egg is "a mere sundering of homogenous materials capable of any fate" is a doctrine which has been given great prominence in recent years. In favor of these commonly accepted views stands a considerable body of experimental work on the development of the ovum; fragments of eggs or isolated blastomeres in many cases are said to give rise to entire larvæ, thus proving, as is usually claimed, that the parts of the egg or embryo are still undifferentiated at the time of the experiment.

But not all experiments on development have confirmed these conclusions; some of the first and most careful researches of this sort led to directly opposite results. In the development of the frog's egg Roux found (1883, 85, 87, 89, 92, 93, 94, etc.) that the median plane of the embryo is determined in the egg immediately after fertilization and that "the development is, from the second cleavage on, a mosaic work of at least four vertical independently developing pieces." In 1887 Chabry showed that the early cleavage cells of the ascidian egg are specified for particular ends and that they develop, if they develop at all, into parts which they would produce under normal conditions. These results were, however, denied on the ground of other experimental work, and by many it was held that the "mosaic theory had received its death blow from the facts of experimental embryology." Among the ova in which isolated blastomeres were found to be capable of complete development were those of ascidians and from this fact it was assumed that here also the early cleavage cells were undifferentiated.

Nevertheless, although some of the results of experimental embryology seemed to render the mosaic theory untenable, additionable evidence in favor of it was furnished by another line of work commonly known as "cell-lineage." In a considerable number of animals belonging to distinct phyla (annelids, mollusks, polyclades, nemerteans, nematodes, rotifers, crustaceans and ascidians) the cleavage of the egg was found to be constant in form and differential in character and each of the early cleavage cells was shown to play a perfectly definite part in the building of the embryo. By those who maintained the traditional view as to the simplicity of the egg and the homogeneity of the blastomeres this fact was explained as due to "the continuity of development," "the position of the blastomeres in the cell complex," etc. But this explanation was never a satisfactory one and is no longer tenable; both observation and experiment have shown conclusively that in certain eggs the blastomeres are not all alike. In particular the experimental work of Crampton (1896), Fischel (1897, 1898), Boveri (1901), Wilson (1903, 1904), Yatsu (1904) and Zelney (1904) has demonstrated that in ctenophores, echinoderms, nemerteans, mollusks and annelids all portions of the egg are not equipotential; this, as well as other work on the organization of the egg, proves that there is a differentiation and localization of the substances of the egg very unlike the "simple undifferentiated protoplasm" of traditional belief.

In most instances the protoplasm of the different blastomeres of an egg is much the same in appearance; in a few cases it is visibly different, but in all cases which have been carefully studied definite blastomeres always give rise to definite parts of the embryo. In fact the manner and rate of development as well as its results are so thoroughly characteristic of certain blastomeres that students of cell-lineage have usually concluded that the protoplasm of different blastomeres must differ, even though these differences are not directly visible. Recent experimental work on some of these forms confirms and extends these conclusions and proves that even in the egg before cleavage begins different substances may be present which are destined in the course of development to enter into specific parts of the embryo.

The most notable differentiations of the oöplasm which have been observed hitherto are found in *Myzostoma* (Driesch, 1896; Wheeler, 1897; Carazzi, 1904), in *Strongylocentrotus* (Boveri, 1901), in *Unio* and *Chætopterus* (Lillie, 1901, 1902), in *Dentalium* and *Patella* (Wilson, 1904) and in the gasteropods *Crepidula*, *Physa*, *Planorbis* and *Limnæa* (Conklin, 1902, 1903). In none of these cases, however, are the differentiations and localizations of the oöplasm so remarkable as are those which occur in the ascidian egg. Here the different substances of the egg are strikingly dissimilar; they are localized in their definitive positions at a remarkably early period, and they may be traced with ease and certainty through the maturation and fertilization, the cleavage, the gastrulation and the later stages until they give rise to specific organs or parts of the larva.

Material and Methods.

I have studied the early differentiations of the egg in three species of simple ascidians, viz., *Cynthia* (*Styela*) *partita* Stimpson, *Ciona intestinalis* (L.) Flemming, *Molgula manhattensis* Verrill. The differentiations and localizations are essentially the same in all of these species, but as the different kinds of oöplasm are more brilliantly colored in *Cynthia* than in either of the other genera named, I shall devote particular attention to this form.

In an extensive publication on the organization and cell-lineage of the ascidian egg (Conklin, 1905), I have figured and described the remarkable localization of germinal materials in the egg of *Cynthia*. The differentiations of the egg substance are here so great and their localization so precise that the figures and descriptions of these might well seem to be exaggerated. I therefore welcome the opportunity of publishing a series of photomicrographs of these eggs, an opportunity which I owe to the skill and courtesy of Misses Foot and Strobell. Their method of photomicrography, which they have fully described in previous publications (Foot and Strobell, 1899, 1900, 1901, 1902) is, in some respects, the most satisfactory ever devised and yet it is as simple as it is complete. The distinguishing feature of this method consists in the accuracy and rapidity with which an exact focus can be obtained; this fact will be better appreciated when the photographs which illustrate this paper are examined in detail and when it is said that thirty such photographs, all of them satisfactory, were taken in less than four hours. With more time and material a more complete series of stages might have been photographed, but this series is sufficiently complete to show the principal features in the differentiation and localization of the egg substances.

All of the photographs are of living eggs in sea water and were taken with a Zeiss Apochromat Obj. 16 mm., Compensating Oc. 4, the bellows length being sufficient to make the magnification 112 diameters. The photographs have not been reduced in the process of reproduction and *neither the negatives nor prints were retouched or altered in any respect whatever.*

The eggs photographed were artificially fertilized and the earliest stage shown (Photo 1) was taken about three quarters of an hour after the sperm were mixed with the ova, but not more than fifteen minutes after the entrance of the spermatozoön into this particular egg. The method of procedure was to place a large drop of sea water containing a considerable number of eggs on a slide and cover with a glass supported by glass "feet" 170 μ thick (the eggs are about 150 μ in diameter). Suitable eggs were then selected and photographed by daylight, the exposure ranging from ten seconds to one minute; the shorter exposure was found to be sufficient while the longer was greatly overtimed (Photos 1 and 13). In order to obtain good color values it is necessary to have the diaphragm rather widely open and this renders the definition somewhat less distinct than it otherwise would be. Furthermore a low magnification was necessary in order to secure sufficient depth of focus to show the entire egg; even with the power employed it was not possible to bring the whole of the egg into good focus at one time. In spite of these evident disadvantages the photographs are really remarkable. Few, if any, other eggs are known in which the organization is so notable as in *Cynthia* and certainly nothing like this has ever before been photographed in the living condition. I wish here to express my great indebtedness to Misses Foot and Strobell for their kindness in making the photographs and also in superintending the production of the plate.

Differentiations and Localizations of the Oöplasm.

In the ovarian egg of *Cynthia* there is a peripheral layer of protoplasm, free from yolk, in which the "test cells" are imbedded and which contains numerous orange-yellow pigment granules which are uniformly distributed. The central portion of the egg consists of yolk-laden protoplasm which is slate-gray in color and lying somewhat eccentrically within the egg is the large, clear germinal vesicle. Before the egg leaves the ovary the chorion is formed and the "test cells" are extruded into the space between the egg and the chorion.

After the egg is laid the germinal vesicle breaks down, but the polar bodies are not formed until after the egg is fertilized. If fertilization does not take place the polar bodies are never extruded, and the clear, the yellow and the gray substances remain in the positions in which they were before the wall of the germinal vesicle disappears. If the egg is fertilized, however, a most astonishing series of movements occur which lead to the localization of the different oöplasmic substances in definite regions of the egg.

The spermatozoön always enters the egg near the vegetal pole and immediately after its entrance the peripheral layer of yellow protoplasm flows rapidly to this pole where it collects as a cap[1] (Photos 1 and 2). At the same time the clear substance derived from the germinal vesicle also flows to the lower pole where it lies between the cap of yellow protoplasm below and the yolk above. This streaming of protoplasm to the lower pole takes place so rapidly that its movements can be directly observed. Within ten or fifteen minutes after the entrance of the sperm into the egg all of the clear and yellow protoplasm has collected at the lower pole while the opposite pole where the polar bodies are formed is at this stage rich in yolk and is slate-gray in color.

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[1] In all the photographs the yellow substance appears very dark, the gray substance less dark while the clear protoplasm is relatively light.

After the clear and the yellow substances have collected at the lower pole the sperm nucleus and aster move toward one side of the egg which future development shows to be the posterior pole ; the clear and yellow substances are also drawn over to this side of the egg and in such a manner that the yellow cap is transformed into a superficial band or crescent which lies just below the equator of the egg on the posterior side, its arms extending forward on each side about half way around the egg (Photos 3 and 4). Owing, perhaps, to the way in which this crescent is formed from the cap of yellow protoplasm its ventral border is sharper and its substance is of a deeper yellow than the dorsal border. At the middle of the yellow crescent is a small area of clear protoplasm which first gathers around the sperm as it enters the egg and which afterward lies at the middle of the crescent throughout the entire development ; this clear protoplasm is seen in profile at the middle of the crescent in Photo 3.

The movement of the protoplasm to the posterior pole is apparently initiated by the movement of the sperm nucleus and aster to this pole ; here the sperm aster divides giving rise to the amphiaster and here the two germ nuclei meet. The axis of elongation of the amphiaster is always at right angles to the axis which connects the animal and vegetal poles while its middle lies in the plane of the first cleavage and in the median plane of the embryo. The amphiaster lies beneath the yellow crescent and some distance from the surface of the egg and the long axes of the two coincide. In Photo 4 the clear line in the middle of the crescent is the amphiaster seen through the superficial layer of the crescent. It is probable that there is some causal connection between the elongation of the amphiaster and the formation of the crescent.

The clear protoplasm which also moves to the posterior pole along with the yellow is chiefly withdrawn from the surface and aggregated around the sperm nucleus and aster, though a portion of it comes to the surface just above (ventral to) the crescent (Photo 3). As the time for the first cleavage approaches the germ nuclei and amphiaster move to the center of the egg and the clear protoplasm goes with them. Finally in the telophase of the first cleavage the clear protoplasm moves into the upper (animal) hemisphere, while the yellow and gray protoplasm are situated in the lower (vegetal) hemisphere.

The first cleavage cuts through the middle of the crescent, the clear protoplasm, and the yolk, the division of all the egg substances being bilaterally symmetrical. *At the close of this cleavage each of these substances occupies its definitive position in the egg (Photos 6–8). The upper clear half of the egg gives rise to ectoderm; the crescent of yellow protoplasm surrounds the posterior side of the egg just below the equator and is later transformed into the muscle and mesenchyme cells of the larva; the gray protoplasm occupies the remainder of the lower hemisphere and gives rise to the endoderm, to the chorda, and to the neural-plate.* Two areas are distinguishable in the gray substance, though I had failed to see them until my attention was called to them by the photographs; the posterior part of the gray material lying in front of the crescent and extending some distance anterior to the vegetal pole is deeper in color and contains more yolk than the anterior portion; the latter forms a light gray crescent around the anterior border of the vegetal hemisphere, just as the yellow protoplasm forms the yellow crescent around its posterior border. The 2-cell stage when seen from the right or left sides (right side in Photo 8) shows all of these areas distinctly, the yellow crescent at the posterior pole (very dark in the photograph), the deep gray material anterior to this, and the light gray crescent occupying the anterior third of the vegetal hemisphere and extending a little above the equator, while the clear protoplasm is located chiefly in the upper hemisphere. The dark gray portion of the vegetal hemisphere gives rise to the endoderm of the larva, the light gray crescent to the notochord and neural plate.[1] *All the principal organs of the larva in their definitive positions and proportions are here marked out in the 2-cell stage by distinct kinds of protoplasm!*

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[1] It is an interesting question whether the chorda-neural-plate crescent of the ascidian egg corresponds to the "gray crescent" of the frog's egg. In both cases it lies on the anterior side of the egg and in the vicinity of the dorsal lip of the blastopore, but it is not positively known that chorda and neural plate are derived from it in the frog, as is the case in the ascidian, though this seems probable.

Although these different oöplasmic substances are chiefly localized in certain regions of the egg, which give rise to certain portions of the embryo, this segregation is not quite complete. Most of the clear protoplasm is found in the upper (ectodermal) half of the egg but some of it is also present in the lower half. Most of the yolk is found in the lower (endodermal) half of the egg, but a little of it is found in the upper half. Almost all of the yellow protoplasm is located in the mesodermal crescent, but a very small amount of it is found around the nuclei of all the cells. Thus samples of all of these egg substances are contained in all of the cells; nevertheless the segregation is so nearly complete that the clear, the gray, the light gray and the yellow areas are marked out with the greatest distinctness (Photos 7, 8).

In the 4-cell stage, as shown by Photos 9 and 10, the distribution of these substances remains as in the 2-cell stage, the yellow crescent being confined to the vegetal hemisphere and the posterior quadrants, the gray crescent to the vegetal hemisphere and the anterior quadrants, while the deep gray, yolk-laden substance lies between these crescents at the vegetal pole and the clear protoplasm occupies most of the animal hemisphere of the egg.

In the 8-cell stage the localization of these substances is the same as in the preceding stages, the clear protoplasm lying above the third cleavage plane and the other substances below it (Photo 11). The perfectly sharp boundaries of the crescent do not coincide with any of the cell boundaries, gray substance being found in the posterior dorsal cells above, below, and anterior to the crescent (Photo 11). The clear notch in the posterior profile of the crescent in Photo 11 is a cap of the same clear protoplasm which gathered around the sperm head at its entrance and afterwards lay at the middle of the crescent (Photo 3). In the 8-cell stage this clear protoplasm takes the form of two caps on the surface of the yellow crescent and adjoining, on each side, the median plane. In this same stage a small amount of yellow protoplasm may be seen around the nuclei of all the cells (Photo 11). This perinuclear yellow substance is most abundant in the posterior-ventral and in the anterior-dorsal cells; in the former it lies chiefly on the dorsal and lateral sides of the nuclei, in the latter on the posterior and lateral sides. In subsequent divisions of these cells this perinuclear plasm retains these positions and therefore goes into certain daughter cells and not into others. Consequently even in those cells in which it is found very sparingly this yellow protoplasm is localized with great definiteness.

In the 16-cell stage all these different substances remain in exactly the same positions which they held at the 2-, the 4- and the 8-cell stages (Photos 12–14). The eight ventral cells are all similar in appearance and are composed chiefly of clear protoplasm. The yellow crescent still surrounds the posterior half of the dorsal hemisphere, as in the 1-cell stage, but it is now contained in four cells; it occupies the posterior and lateral portions of these cells while the anterior and median portions are composed of gray substance. The boundary between these two substances could not be sharper if they were actually, as at first sight they seem to be, separated by a cell wall (Photo 14); only at the next stage, however, are these substances segregated into separate cells. The gray crescent occupies the anterior portions of the four anterior cells of the dorsal hemisphere, the posterior portions of these cells being composed of deep gray (endodermal) substance (Photo 14).

At the 32-cell stage (Photos 15, 16) the substances of the yellow and the gray crescents are finally segregated into separate cells, although a small portion of deep gray substance is still contained in the median cells of the yellow crescent; however this gray material moves in from the surface so that it does not show in photographs of the entire egg. The yellow crescent now consists of six cells, four median ones which are small and one pair of lateral ones which are relatively large (Photos 15, 16). The gray crescent consists of four cells of equal size and similar constitution. The ventral half of each cell is clear, contains little yolk and gives rise to most of the neural plate, the dorsal half is light gray in color, is yolk-laden and gives rise to the chorda. From this stage onward the ventral hemisphere is composed of clear cells, all of which are much alike; for this reason no photographs are given of the later stages of these cells.

In subsequent stages all of the cells of the gray and yellow crescents divide in a vertical direction (parallel to the egg axis); these divisions occur first in the gray crescent and in the most anterior pair of cells of the yellow, then in the pair of yellow cells adjoining the latter behind, and finally in the posterior median pair. The subdivision of the cells of the gray crescent occurs at the 44-cell stage; the ventral product in each case is a small clear cell which ultimately forms the posterior portion of the neural plate, the dorsal products are larger and are gray in color and ultimately develop into the chorda.

In the case of the yellow crescent both products of the posterior median cells give rise to mesenchyme, but the ventral ones contain those caps of clear protoplasm which were visible in the 8-cell stage (Photo 11) and which in a still earlier stage first appeared around the entering spermatozoön (Photo 3). The dorsal products of the other two pairs of crescent cells give rise to mesenchyme, while from the ventral halves come the muscle cells of the tadpole's tail. The mesenchyme cells are clear and faintly yellow in color, the muscle cells are a deep yellow and these two substances are distinguishable in their definitive positions even as early as the 1-cell stage (Photo 3).

Unfortunately no photographs of the early stages of gastrulation were taken. I have however studied and drawn every step of this process both in living and in prepared material. Gastrulation begins with the depression of the endoderm cells just posterior to the chorda cells, and is later continued by the rolling in of cells around the margin of the blastopore. In this manner the neural plate cells come to overlie the chorda cells, and the muscle cells, the mesenchyme.

In the closure of the blastopore the posterior (ventral) lip remains stationary until the last stages of the process, while the anterior (dorsal) lip grows backward over the gastrocoel until the blastopore is reduced to a longitudinal groove between the muscle cells of each side (Photo 17). In the overgrowth of the dorsal lip the rows of muscle cells as well as the blastopore groove are forced to the hinder end of the embryo and the muscle rows are tilted up at their anterior ends until they are transverse to the long axis (Photo 17). Later the ventral lip overgrows the remnant of the blastopore and the ectoderm of this lip forms a pair of V-shaped folds which fuse from behind forward and thus cover the dorsal lip and roll the neural plate up into a tube. In this overgrowth of the ventral lip the transverse rows of muscle cells again assume an antero-posterior direction in the embryo (Photos 18–21).

Since the neural plate is composed of relatively transparent cells which overlie the gray chorda plate, it is not well shown in the photographs, unless seen in profile (Photos 22, 23). In the dorsal view shown in Photo 18 seven or eight transverse rows of cells may be indistinctly seen in the neural plate. The chorda plate, which contains considerable yolk and is gray in color lies under the neural plate. It consists at first of a single transverse row of eight cells, then by the division of these cells two such rows are formed and finally by shoving together from the sides this plate becomes much narrower and longer. In later stages the chorda plate and the neural plate push back between the muscle cells of each side until they reach the hinder end of the embryo, thus establishing the characteristic appearance of the young larva shown in Photo 21. It can now be seen distinctly that the deep yellow cells have become the lateral muscles in the tail, that the light gray cells of the chorda plate have formed the fusiform chorda which lies between the muscle cells, and that the deep gray cells form the gastral endoderm. At the posterior end of the chorda is a group of light yellow cells which connect the muscle rows of the right and left sides; these are the caudal mesenchyme cells. At the anterior ends of the muscle rows are the clear areas of the trunk mesenchyme, which are also of a faint yellow color, while around the entire embryo is a clear layer of ectoderm cells (Photo 21).

The form of the larva is now well established and subsequent development changes this form only in minor features. In Photo 23 the tail is much elongated and bent toward the ventral side. Three rows of muscle cells with clear nuclei can be seen on the left side of the tail, while the larva is tilted toward the left so that the dorsal row of muscle cells of the right side is also visible; with the elongation of the tail the individual muscle cells have become much longer than in previous stages. Between the dorsal rows of muscle cells of the right and left sides is a clear line which is the neural tube; anteriorly this tube overlies the dark gray endoderm and hence it is not clearly visible in the photograph, but its anterior end appears as a clear, triangular area, notched where the tube is still open at the anterior end of the larva. This clear, transparent condition of the nervous system, both in the tail and trunk regions shows that the yellow protoplasm does not enter into its formation and that the muscle cells are not "neuro-muscular" cells as claimed by Castle (1896). In this and the following photograph (Photo 24) the larva is well developed, though the sense organs have not yet appeared in the sense vesicle. The most important organs of the larva are here clearly recognizable in the photographs of the living tadpoles, viz., the muscles, the notochord, the central nervous system, the gastral endoderm, the caudal and trunk mesenchyme and the ectoderm. *The substance of each of these organs is peculiar in color and constitution and these different substances may all be traced back to the 2-cell stage, where they occupy positions corresponding to their ultimate locations in the larva, while the substances of the ectoderm mesoderm and endoderm are recognizable in the unsegmented egg.* With the exception of the early gastrula stages, which were not photographed, every important step in the transformation of these substances into the organs named can be followed in the photographs of the living eggs and embryos!

NATURE AND POTENCY OF THE OÖPLASMIC SUBSTANCES.

The fact that definite blastomeres of the ascidian egg give rise to definite portions of the larva has long been known (Van Beneden and Julin, 1884; Castle, 1896). Furthermore Chabry (1887) found that when certain blastomeres were killed the remaining ones gave rise only to a partial larva. On the other hand, Driesch (1895, 1903) and Crampton (1897) found that individual blastomeres of the ascidian egg developed into entire larvæ. The mere observation of the egg of *Cynthia* shows that certain areas are marked out from the time of fertilization, or even earlier, by distinct kinds of protoplasm and that these areas give rise in the course of normal development to definite organs. But, in view of the work of Driesch and Crampton, by what right are these areas called organ-forming regions and what is the justification for calling the substances of these areas organ-forming substances? The answer to both of these questions is the same, viz., in the absence of a region or substance, the organ to which it would normally give rise is not produced; and conversely each substance develops, if it develops at all, into the parts which it would normally produce. Experiments which I have carried out on ascidian eggs[1] show that the development of isolated blastomeres is strictly partial, as was first shown by Chabry and afterwards denied by Driesch. As yet I have been unable to get the isolated substances of the unsegmented egg to develop at all, but when they are isolated during the cleavage stages they develop only into the parts which they would normally produce, while the portions of the egg or embryo which lack these substances develop into embryos which lack the corresponding organs. Since the first cleavage of the egg is bilaterally symmetrical and divides all the substances of the egg equally, each of the first two blastomeres contains one half of all the organ-forming regions and substances; and since isolated blastomeres of the ascidian egg always produce rounded masses of cells which tend to close over the injured surface, many of these half embryos have the appearance of whole embryos of half size; but a careful study of living material as well as of stained preparations and sections shows that the larvæ are still incomplete up to the time of the metamorphosis. When the division of the egg or embryo is made along any other plane than the median one nothing even remotely resembling a normal larva is obtained. Every substance of the egg develops, if it develops at all, into the organs which it would normally produce, and while it has not been possible to isolate these substances in the unsegmented egg, their appearance is the same before and after cleavage begins and under these circumstances there is small room for doubting that even in the unsegmented egg these are actually organ-forming substances.

Therefore in the unsegmented egg and early cleavage stages of *Cynthia partita* we have the most complete differentiation and localization of the oöplasm ever yet reported for any egg. Apart from the nuclei, the centrosomes and the asters, there are visible in the 2-cell stage six different kinds of cytoplasmic substance,

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[1] These experiments will be published in full elsewhere.

each of which gives rise to some specific portion of the larva and is here present in its definitive position and proportions, viz., the clear protoplasm which gives rise to the ectoderm, the gray substance which produces endoderm, the deep yellow substance which develops into the muscle cells, the light yellow which goes into the mesenchyme, the clear protoplasm at the middle of the yellow crescent which becomes caudal mesenchyme and the light gray substance of the gray crescent which gives rise to chorda and neural plate. Inasmuch as it is difficult to refer to these different substances of the egg by the purely descriptive terms which have been employed thus far, I propose to designate them by names suggestive of the parts to which they ultimately give rise, viz., ectoplasm, endoplasm, myoplasm, chymoplasm, caudal chymoplasm and chorda-neuroplasm.[1] Of all of these substances the mesoplasm (myoplasm and chymoplasm) alone takes its definitive position before the first cleavage; the other substances reach their final positions only at the close of this cleavage. But although the localization is not complete in the unsegmented egg the ectoplasm and endoplasm are nevertheless clearly differentiated before cleavage begins; I am unable to say whether the chorda-neuroplasm is also differentiated at this stage.

CLEAVAGE AND GERMINAL LOCALIZATION.

In the early stages of development it is apparent that the cleavage planes do not closely follow the lines of separation between the different substances of the egg. The yellow crescent is bisected at the first cleavage; the second cleavage passes anterior to it; the third cleavage plane lies some distance above (ventral to) the upper border of the crescent; the fourth cleavage bisects the halves of the crescent on each side of the median plane. No one of these first four cleavage planes follows any one of the boundaries of the crescent; the same is also true of all the other oöplasmic substances. Although the localization of these substances is precise and definite, the localization pattern does not correspond to the early cleavage pattern. In the later cleavages some of the division walls do closely correspond with the planes

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[1] The substances of the chorda and neural plate are not clearly distinguishable from each other before the 8-cell or 16-cell stage.

of separation between these substances so that these various substances are ultimately segregated into definite cells, but this perfectly definite type of localization arises without reference to cell division and is not appreciably altered by subsequent divisions. During the first cleavage the yellow crescent substance may be seen to be undergoing complex vortical movements, but this does not permanently change the form or position of the crescent. In the unsegmented egg and in all the subsequent stages of the cleavage the yellow crescent occupies its initial position on the posterior side of the egg below the equator, irrespective of the position of the cleavage planes. Likewise the gray crescent, the ectoplasm and the endoplasm occupy the same positions in the egg at the beginning of gastrulation as in the 2-cell stage; in fact so far as localization is concerned the condition at the close of cleavage is the same as at its beginning.

Cytoplasmic and Nuclear Organization.

All of these different organ-forming substances are present, and are shown in the photographs, as early as the close of the first cleavage, some of them much earlier. In fact the clear ectoplasm, the gray endoplasm and the yellow mesoplasm are recognizable in the ovarian egg. Here the mesoplasm forms a peripheral layer around the whole egg in which the "test cells" are imbedded, the ectoplasm is contained within the large germinal vesicle, while the endoplasm occupies the remainder of the cell. Tracing these differentiations still further back it is found that at least a portion of the mesoplasm comes from the sphere substance (archoplasm), which is probably derived in part from the nucleus of the last oögonic division (Conklin, 1902). The yolk also is formed by the activity of the "yolk matrix" (Crampton, 1899) or yolk nucleus which is probably derived from the sphere substance. Portions of the ectoplasm, mesoplasm and endoplasm [1] are thus derived from the nucleus, the first from the nucleus of the oöcyte, the last two from the nucleus at the last oögonic division.

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[1] It may of course be objected that the yellow, the gray, and the clear substances of the ovarian egg have not been proven to be differentiated for particular ends and this I freely grant to be the case. Furthermore I do not see how this question could be tested experimentally, especially in the case of the immature ovarian egg. The fact that these substances are visibly different from one another in the oöcyte and that in all respects they resemble the ectoplasm, mesoplasm and endoplasm of the cleavage stages, to which they ultimately give rise, is the only reason for continuing to call them by these names in this earlier stage.

This remarkable condition in which considerable portions of the oöplasm can be traced back to the nucleus is of the greatest theoretical importance. From all sides the evidence has accumulated that the chromosomes are the principal seat of the inheritance material until now this theory practically amounts to a demonstration. On the other hand all persons who have much studied cell-lineage have been impressed with the fact that polarity, symmetry, differentiation and localization are first visible in the cytoplasm and that the positions and proportions of embryonic parts are dependent upon the location and size of certain blastomeres or cytoplasmic areas. However in the fact that large quantities of "nuclear sap" containing dissolved oxychromatin escape into the cell body at every mitosis (*v.* Conklin, 1902) and that these nuclear substances then contribute to the formation of specific organ-forming substances of the cytoplasm we see a possible means of harmonizing the facts of cytoplasmic organization with the nuclear inheritance theory.

Types of Germinal Organization.

By those who maintain the view that the egg is typically composed of "simple undifferentiated protoplasm" the remarkable organization of the ascidian egg will probably be regarded as an extreme case of precocious differentiation. This may perhaps be the case but the fact that germinal differentiations and localizations occur in the eggs of annelids, mollusks, nemerteans, echinoderms, ctenophores, nematodes and ascidians shows that it is by no means a rare phenomenon and it really seems as if the burden of proof were shifted to those who maintain that the egg is typically undifferentiated. Unquestionably the egg is less highly differentiated than the embryo or larva, as organ-forming substances are simpler than the organs to which they give rise, but the evidence drawn both from observation and experiment shows conclusively that in a large number of animals the substances of the egg are not homogeneous nor equipotential. But even granting that there are cases in which there is no such differentiation of the oöplasm, this supposed lack of differentiation can apply only to portions of the egg, the cytoplasm for instance, for of course there must be determinative factors (" determinants ") somewhere in the ovum, probably in the nucleus which differ from one another in kind.

However in the phyla named the localization of morphogenic substances in the cytoplasm is sufficiently definite to warrant a comparison of one group with another. In all the ascidians which I have studied and apparently in all which have been studied hitherto, the type of localization is the same. Furthermore there is good reason for supposing that this type is essentially like that of *Amphioxus* and Amphibia (on this subject see Conklin, 1905). Judging from the work which has been done on the organization of the egg in other phyla, this chordate type is very distinct from that of annelids, mollusks, nemerteans, echinoderms, nematodes or ctenophores. In fact it seems necessary to recognize several distinct types of localization.

If one has regard only to the localization of the substances of the germinal layers there is considerable uniformity among most metazoa in their pregastrular stages. In almost all cases the ectodermal substances are localized in that hemisphere of the egg which is nearest the polar bodies, and in this the ascidians are no exception to the rule; in many cases the mesodermal substances are at first localized at the opposite pole, though only among the echinoderms (*Strongylocentrotus*, Boveri, 1901) is this localization persistent; among annelids, mollusks, and ascidians the mesodermal substances early move from this pole to the posterior side of the egg.

However in the localization of specific organ bases there are many notable differences among these phyla. In this regard the annelids and mollusks and perhaps the nemerteans belong to one type, the chordates, nematodes and ctenophores to entirely different types; in fact the localization of organ bases in the ascidian egg does not resemble that in the other phyla named any more closely than does the localization of the larval or adult organs of these phyla; indeed, the principal chordate features are already represented in the ascidian egg by characteristically localized organ bases as early as the 2-cell stage.

Since the time of Cuvier the principal criterion of homology has been, in the words of Owen, "correspondence in the relative position and connexion of parts." Such correspondence has been found to be much more fundamental than resemblances in size, proportions, or details of structure. Similarly in germinal organization it seems probable that the relative positions and connections of organ bases are essentially alike in different members of a phylum, though in other respects, they may vary widely. A peculiar type of localization of organ bases is thoroughly characteristic of the ascidian egg and probably the same thing is true of other phyla. If this be true, different phyla do not approach one another more closely in the earliest stages of germinal localization than in the cleavage or gastrular stages.

ORIGIN AND EVOLUTION OF GERMINAL ORGANIZATION.

The fact that there are various types of germinal localization corresponding to different types of adult organization will be explained by most persons as due to the gradual "acceleration" of development, or the shifting of adult characters back to earlier and earlier stages of the ontogeny. It is but natural that those whose attention is focused upon adult structures should regard the adult as primary, the germ as secondary; but it is surprising that embryologists also have almost universally held a similar view. Even students of cell-lineage and of the organization of the egg have generally regarded this organization as secondary and have explained it as the result of "precocious segregation," or of the "reflection of larval or adult characters back upon the egg."

Such conclusions are not founded upon observation nor experiment but upon preconceived notions as to the importance of the adult and the extreme simplicity of the germ. The whole life cycle is commonly viewed from the standpoint of the adult, and all other stages are supposed to exist *for the purpose* of leading up to a definite end stage. Similarly evolution is looked upon as the transmutation of definite end stages into others by direct modification of certain adult structures, which in some way or other modify the germ and thus become inherited.

But what is the evidence that, in either ontogeny or phylogeny, the adult is primary and the germ secondary? What is the ground for supposing that evolutionary changes first occur in the end stages and only later affect the earlier stages in the life cycle? In spite of the age-long controversy as to the inheritance or non-inheritance of acquired characters there is no satisfactory evidence that particular modifications of any adult part ever produce specific modifications of the germ. All the evidence available seems to show that the soma stands to the germ in the relation of environment and that the only influence exercised by the former upon the latter is of a general character, as Weismann has so ably argued.[1] Furthermore the difficulty of conceiving of any method by which adult characters might be transferred to the germ is well known. No hypothesis ever yet proposed for the solution of this problem harmonizes with the established facts of oögenesis and spermatogenesis. If such transfer occurs, of which there is no sufficient evidence, it can only take place by methods of which we are at present wholly ignorant.

On the other hand there is much to be said in favor of the view that the germ is primary, the adult secondary and that heritable modifications first arise in the germ and only later appear in the adult. Apart from the fact that the germ gives rise to the adult and to other germs, it is known that in certain cases apparently slight modifications of the germ may produce profound modifications of the adult, whereas the reverse is not known to be true. One of the most convincing evidences of the truth of this view is found in cases of cross breeding, particularly in hybridization, where it is certain that hybrid characters of an offspring are directly due to the hybrid character of the germ, since they can have no other possible cause. The evidence drawn from experiments on eggs and embryos on first thought seems to be conflicting; in some cases fragments of eggs or embryos give rise only to partial larvæ and injured eggs produce only embryos showing more or less serious defects; in other cases entire embryos are produced under these conditions, but these results cannot be regarded as destructive of this argument for, as has long been maintained by Roux, such cases of entire

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[1] Undoubtedly one important cause of germinal variation is to be found in the influence of changing environment upon the germ, but this is far from saying that particular modifications of the adult are transmitted to the germ.

development of egg fragments may be the result of regenerative, or regulative, processes. It is not usually possible to connect definite modifications of the adult with definite alterations of the germ from which it developed, but one remarkable instance in which this is possible is found in cases of inverse symmetry. In sinistral gasteropods, and presumably in all other cases of inverse symmetry, the cause of inversion is to be found in the inverse organization of the unsegmented egg and I have elsewhere (1903) shown reason for believing that this may be due to the maturation of the egg at opposite poles in dextral and sinistral forms. Here one of the most sudden and profound alterations of structure with which we are acquainted may be traced back to a specific modification of the germ.

These facts point to the conclusion that the complex organization of an egg, such as that of an ascidian, has not arisen through the "reflection of adult characters upon the egg," but rather that this organization is primary. Furthermore they seem to indicate that evolution has taken place, not through modifications of adult structure, but through changes in germinal organization; modifications of this organization, however produced, are probably the real causes of evolution.

This conclusion, which has grown out of a study of the complex organization of the germ and its relation to adult organization, harmonizes entirely with the mutation theory of DeVries; it indicates how mutations in elementary germinal characters may appear as widespread modifications in the mature organism; it offers an explanation of otherwise inexplicable variations of adult structure, such as inverse symmetry; and finally it suggests a possible solution of that vexed problem of the origin of phyla, not by the transmutation of one adult form into another, as is assumed in all previous hypotheses, but by relatively simple alterations of the type of germinal organization.

University of Pennsylvania, Philadelphia,
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DESCRIPTION OF PHOTOGRAPHS. (Plate XI.)

All the photographs are of the living eggs and embryos of *Cynthia* (*Styela*) *partita* in sea water and are magnified 112 diameters. Prints from the original negatives were arranged in order and photographed and the plate is an actual print (Rotograph Process) from this negative. These photographs are not diagrams but like the specimens themselves they require and will repay careful study. That portion of the egg which appears darkest in the photographs is the orange-yellow mesoplasm, that which is lightest is the transparent ectoplasm, while that which is apparently intermediate in shade between these two is the slate-gray endoplasm. The contrast between these substances is therefore greater in reality than appears in the photographs. Every egg or embryo is inclosed in a transparent chorion which does not show in the photographs; within the chorion and around the periphery of the egg are numerous "test cells" which contain yellow pigment.

Photo 1. Egg about fifteen minutes after the entrance of the sperm showing the yellow protoplasm as a dark cap at the lower pole where the sperm lies. The clear protoplasm is the light zone above the yellow; the rest of the egg is gray.

Photo 2. Egg about twenty minutes after the entrance of the sperm; the egg substances are shown as in the preceding photograph.

Photo 3. Egg about thirty minutes after the entrance of the sperm, seen from the right side. The yellow protoplasm is moving to the posterior pole and forming the crescent there; the clear protoplasm lies chiefly above (ventral to) the crescent; in the middle of the crescent and at the periphery of the egg is a small spot of clear protoplasm (caudal chymoplasm) which first appears around the entering sperm and ultimately goes into a pair of caudal mesenchyme cells.

Photo 4. Egg about thirty-five minutes after the entrance of the sperm, showing the yellow crescent as a dark band with a clear area through its center; the latter is the first cleavage spindle. Both the crescent and the periphery of the egg show a slight notch in the lower (ventral) border, which is the beginning of the first cleavage furrow. The egg is somewhat obscured by overlying test-cells which here and elsewhere give it a mottled appearance; high focus.

Photo 5. Egg about forty minutes after the entrance of the sperm, viewed from the posterior ventral pole. The cleavage furrow is deepest in the region of the crescent. Above the crescent is the clear (ventral) ectoplasm. High focus.

Photo 6. Egg about forty-five minutes after the entrance of the sperm. Two-cell stage viewed from the animal pole showing the yellow crescent at the posterior margin of the egg.

Photo 7. Stage similar to the preceding, viewed from the posterior pole; below the crescent is shown the gray endoplasm of the vetegal pole, above the crescent the clear ectoplasm of the animal hemisphere, in the furrow at the middle of the crescent a small amount of clear chymoplasm.

Photo 8. Stage similar to the preceding, viewed from the right side (an end view of one of two cells), showing the clear ectoplasm in the upper (animal) hemisphere, the yellow crescent (mesoplasm) at the posterior pole, the light gray crescent (chordaneuroplasm) at the anterior pole and the dark gray endoplasm between the two crescents at the lower pole. The definitive localization of these substance is complete at this stage.

PHOTO 9. Four-cell stage from vegetal pole, showing the yellow crescent across the two posterior cells. The anterior cells lie at a lower level than the posterior ones and the focus is such that only the ends of the crescent show clearly.

PHOTO 10. Four-cell stage from the animal pole, high focus; many test cells cover the egg; the yellow crescent, which lies on the lower side of the posterior cells, shows indistinctly through the egg; an area of clear protoplasm is shown in each of the cells.

PHOTO 11. Eight-cell stage from the right side; the upper cells contain the clear ectoplasm, though a small amount of yolk is found at the periphery of each cell; most of the yellow protoplasm is contained in the yellow crescent, the outline of which is very distinct, but a small amount of yellow protoplasm is found around the nuclei of all the cells; in the posterior ventral (upper) cells this lies on the lateral and dorsal side of the nucleus, in the anterior dorsal (lower) cells it lies on the lateral and posterior side of the nucleus. At the middle of the yellow crescent and seen as a notch in its posterior outline is a small cap of caudal chymoplasm (the same as that seen in Phôto 3). The yellow crescent is bounded by dark gray endoplasm which extends forward to the middle of the anterior-dorsal cells; the gray crescent of chorda-neuroplasm occupies the anterior portions of these cells. The forward slant of the vertical (second cleavage) furrow and the "cross furrow" formed by it and the third cleavage are clearly shown. Photos 3, 8, and 11 are all viewed from the right side and the localizations of the same organ-forming substances in the 1-, 2- and 8-cell stages are clearly shown in these photos.

PHOTO 12. Sixteen-cell stage from dorsal side, view slightly oblique. The eight dorsal cells are clearly shown, while three transparent ventral cells are indistinctly shown on the left-anterior periphery. The yellow crescent is contained in the four posterior cells, the lighter margins of the four anterior cells represent the gray crescent. The median cells behind are nearly filled with yellow mesoplasm, save for the clear nuclei and a small wedge of gray substance in the anterior portion of these cells; the lateral portions of the cells just anterior to these are composed of yellow substance, their median portions of gray material.

PHOTO 13. Sixteen-cell stage from the posterior pole, showing the four yellow crescent cells with clear nuclei; below them is the gray endoplasm, above them the clear ectoplasm; the ectoderm cells are indistinctly shown with a trace of yellow substance around nuclei of the four posterior cells.

PHOTO 14. Sixteen-cell stage transitional to 32-cell stage, dorsal view showing eight cells. The localization of the different oöplasmic substances is the same as in Photo 12, but the focus is a little deeper. The yellow and gray crescents are remarkably distinct; between the two is the area of deep gray endoplasm; the light area on the inner border of the yellow crescent is chymoplasm.

PHOTO 15. Thirty-two cell stage, dorsal view. The small posterior crescent cells have divided transversely, forming four small cells; the large mixed cell anterior to these is just cutting off its outer yellow portion from its inner gray one. The four anterior cells have divided in an antero-posterior direction thus separating the gray crescent of chorda-neuroplasm from the endoplasm. The nuclei in all the cells appear as clear areas.

PHOTO 16. Thirty-two cell stage, dorsal view, similar to the preceding.

PHOTO 17. Advanced gastrula, posterior view, superficial focus. The blastopore groove is a narrow slit bounded on each side by four large muscle cells, which are derived from the yellow crescent ; the lighter colored cells at the bottom of the groove are mesenchyme and are derived from the middle part of the crescent. The dorsal lip of the blastopore (not clearly shown in the photo) closes the groove dorsally and anteriorly.

PHOTO 18. Late gastrula, dorsal view, superficial focus. The U-shaped group of mesoderm cells (Photo 17) is seen from the open end of the U ; the ectoderm overgrowing the mesoderm is seen as a light area posterior to the yellow cells ; the light wedge-shaped area in the mid-line is the beginning of the neural groove ; the apex of the wedge lies between the limbs of the U and marks the point at which the blastopore closes and also the posterior limit of the neural plate. The ectoderm can be seen as a zone of clear cells with transparent nuclei around the periphery and faint indications of these cells with their clear nuclei can be seen forming seven or eight transverse rows of cells across the embryo anterior to the blastopore (the neural plate). The gray endoderm seen through these ectoderm cells gives a dark appearance to all the embryo save the periphery.

PHOTO 19. Late gastrula, dorsal view, deep focus. This stage is later than the preceding and the embryo is tilted slightly toward the right side, so that the plane of symmetry is a little to the right of the middle of the photo ; the mesoderm cells are no longer transverse to the long axis but are extending in an antero-posterior direction. In front of the mesoderm is a dark area, the endoderm, in which four transverse rows of cells may be indistinctly seen ; around the entire periphery is the clear ectoderm. The anterior portion of the embryo is wider and the posterior part narrower than at any previous stage.

PHOTO 20. Young tadpole, ventral view, superficial focus ; the larva is slightly tilted to the right so that the ventral mid-line lies to the right of the middle of the photo. Three rows of rounded muscle cells (six or seven cells in a row), with clear nuclei, lie on each side of the mid line. In front of the muscle cells on each side is a clear area of mesenchyme. The strand of caudal endoderm cells shows in the mid-line between the muscle rows of each side ; anterior to the muscle and mesenchyme is the gastral endodern.

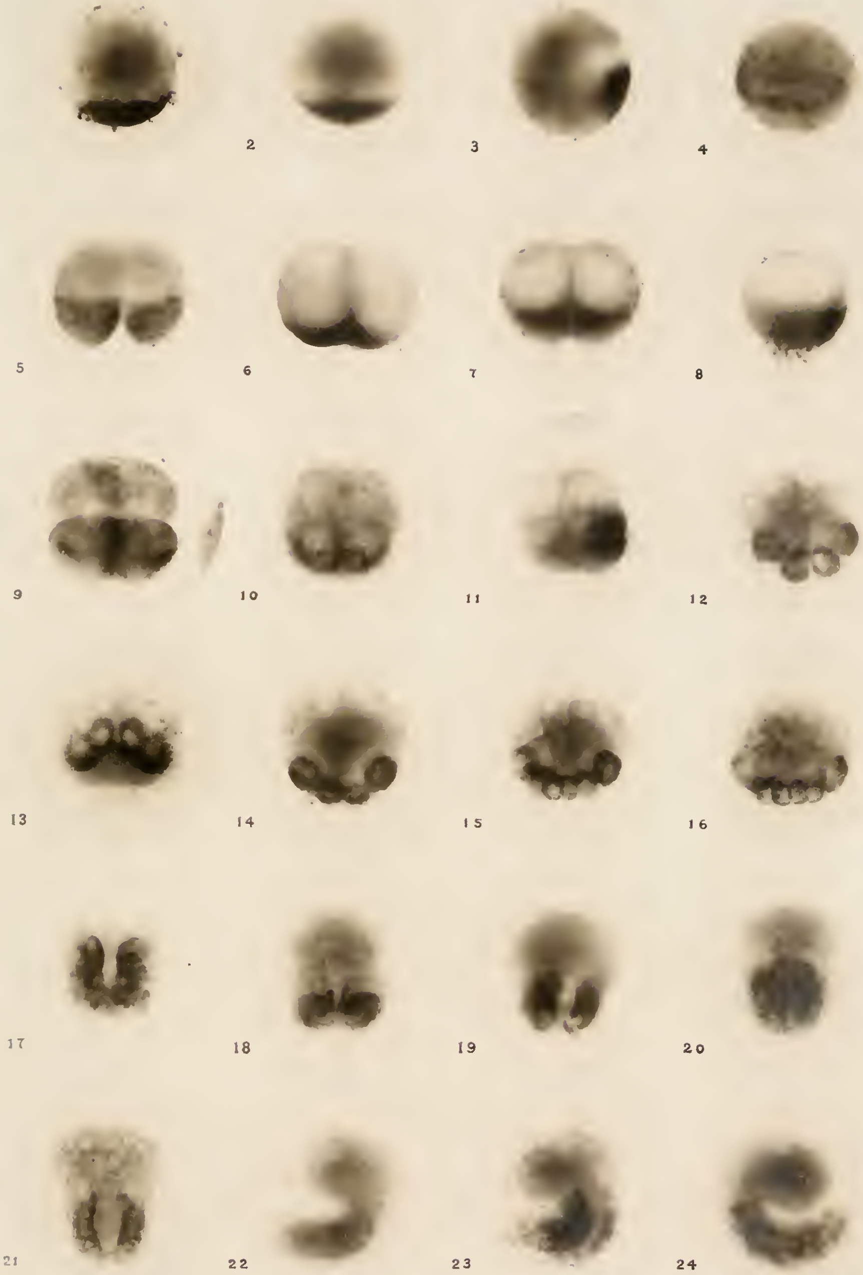
PHOTO 21. Young tadpole, ventral view, deep focus. Between the muscle cells is tbe fusiform notocord which is composed of wedge-shaped cells. Six muscle cells are visible on each side of the chorda and there are several lighter colored mesenchyme cells at its posterior end. At the anterior ends of the muscle rows is a clear area of mesenchyme cells in which the peribranchial pouches appear. Cells of the gastral endoderm are clearly visible ; around the entire periphery are clear ectoderm cells.

PHOTO 22. Young tadpole from left side. The three rows of muscle cells, each with a clear nucleus are faintly shown in the dark area in the tail, the gastral endoderm also appears as a dark area in the trunk, but not so dark as the muscle cells. Around the entire periphery is the clear ectoderm ; on the dorsal (convex) side this is especially thick and comprises the neural tube. The hazy areas ventral to the embryo are due to aggregated test cells.

PHOTO 23. Tadpole of about the same stage as the preceding, left-dorsal aspect.

The rows of muscle cells show clearly along the left side ; the dorsalmost row of the right side also shows and between these two is a clear line, the nerve tube (or cord); anteriorly this cord leads to the wedge-shaped light area at the anterior border of the larva, which is the sense vesicle (brain) and which is still open to the exterior. The test cells somewhat obscure the posterior portion of the tail.

PHOTO 24. Tadpole a little older than the preceding viewed from the right side. The tail is much elongated and the muscle cells, each faintly marked by a clear nucleus and dark outline, are also elongated as compared with preceding photos. Two rows of muscle cells are distinctly visible, the third also comes into view at the hinder end of the tail. There are seven or eight cells in each row as indicated by the number of nuclei. The gastral endoderm shows as a dark area in the trunk. The neural cord and tube is the clear area on the dorsal side of the trunk, its anterior limit being marked by a flattened contour line where the neural folds are just closing.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CONKLIN. ORGAN-FORMING SUBSTANCES.

RESEARCH SEMINAR OF THE MARINE BIOLOGICAL LABORATORY.

1. Zoölogy, Season of 1904.

July 5. **Work on the Life Histories of some Cestode Parasites of Fishes.** By Winterton C. Curtis.

July 8. **Conditions that Determine the Relative Position of Chromosomes in Mitosis.** By Ralph S. Lillie.

July 12. **The Physiology of the Invertebrate Heart.** By A. J. Carlson.

The following points were demonstrated by drawings and graphic records:

1. With the exception of *Mytilus*, in which the results are not conclusive, the auricles and the ventricle of the lamellibranchs (*Mya*, *Tapes*, *Venus*, *Hennites*, *Pecten*, *Cardium*) are supplied with inhibitory nerves from the visceral ganglion or ganglia. These fibers reach the heart in the renal nerves and enter at the base of the auricles. No nerves enter the heart along the aortæ or the rectum.

The heart of the prosobranchs (*Haliotis*, *Lucapina*, *Natica*, *Sycotypus*) and the tectibranchs (*Aplysia*, *Bulla*, *Pleurobranchæa*) is supplied with accelerator nerves from the visceral ganglion or ganglia. These nerves enter the ventricle at the aortic end. Some fibres may also reach the ventricle through the auricular walls. The auricular nerves enter the auricle at its base.

The heart of the nudibranchs is supplied with regulative nerves from the brain or supraœsophageal ganglion. In *Montereina* these nerves appear to be of the accelerator type only, while in *Triopha* both inhibitory and accelerator nerves are present.

The auricle of the slugs (*Limax*, *Ariolimax*) and the snail (*Helix*) is supplied with both inhibitory and accelerator nerves from the subœsophageal or pleural ganglion. These nerves enter the auricular musculature at the base of the auricle. The innervation of the ventricle of these pulmonates appears to be less uniform. The ventricle of *Helix* is supplied with both inhibitory and accelerator fibres from the same nerve as the auricle. These fibers enter the ventricle at the aortic end, some fibers reach the ventricle also through the walls of the auricle. The ventricle of *Limax* appears to be supplied with inhibitory nerves only, which enter it through the auricular walls. The ventricle of *Ariolimax* is supplied with accelerator, and probably also inhibitory fibres from the same source as the auricle. The fibres enter the ventricle at the aortic end. In *Helix* the influence of the inhibitory fibres on the ventricle, in *Ariolimax* that of the accelerator, is the greatest.

The systemic and the gill hearts of the cephalopods (*Octopus*, *Loligo*, *Ommastrephes*) are supplied with inhibitory fibres from the visceral nerves, and there is some evidence that accelerator fibres reach these hearts from the same source. In the squid the fibres to the systemic ventricle and to the auricles leave the visceral nerves at different levels. The rhythmically contracting parts of the renal veins are probably supplied with inhibitory fibres from the visceral nerves. The visceral nerves also send fibres to the pulsating vena cava. Their function was not made out.

The heart of crustaceans is supplied with inhibitory and accelerator fibres from the thoracic ganglion. In *Palinurus* the inhibitory and the accelerator fibres reach the heart along two separate pairs of nerves.

The heart of *Limulus* is supplied with inhibitory nerves from the posterior end of the brain or periœsophageal ganglion, and with accelerator nerves from the abdominal ganglia. These nerves enter the nerve-cord on the dorsal side of the heart. There is some evidence that the heart of spiders and insects is supplied with inhibitory nerves from the brain or the thoracic ganglia.

2. The action of the inhibitory nerves on the invertebrate heart is the same as the action of the vagus fibers on the vertebrate heart. The accelerator nerves in the molluscs produce contractions in the quiescent heart. Single induced shocks applied to the cardiac nerves are usually without influence on the heart unless of considerable intensity.

3. The latent period of the heart-muscle is less than 0.03″ in

the lamellibranchs and the gasteropods; in the cephalopods it does not exceed 0.02″; in the crustaceans it does not exceed 0.01″. The latent period of the heart-muscle is not greater than that of the body-muscle of the same animal.

4. The rate of propagation of the contraction in the tunicate heart (*Ciona*) varies from 200 to 350 mm. per sec.

5. The molluscan, the arthropod, and the tunicate heart exhibits a condition of reduced excitability at the beginning of systole, but no refractory period in the sense of inexcitability.

6. The molluscan, the arthropod, and the tunicate heart, that is greatly fatigued or injured, and in poor condition so that it does not beat spontaneously, responds to stimuli of increasing intensity with contractions of increasing amplitude; but the pulsating heart or the quiescent heart whose excitability is not greatly reduced tends to respond with contractions of uniform amplitude to stimuli of increasing intensity within a wide range, but increasing the strength of the stimulus above this range is followed by supermaximal contractions. The "all-or-none" law does not apply.

7. In the molluscan and the crustacean heart a strong induced shock sent through the heart at the beginning of systole diminishes the strength of that beat.

8. The molluscan, the arthropod, and the tunicate heart is inhibited in diastole by the interrupted current of a certain intensity applied directly to the heart. This inhibition is obtained in hearts apparently not provided with inhibitory nerves, and in hearts provided with inhibitory nerves after these have been paralyzed by the action of drugs. It is therefore an action of the induced current directly on the rhythmical tissue.

9. The molluscan, the arthropod, and the tunicate heart can be tetanized.

July 15. Feeding Experiments for Determining the Life History of an Oyster Parasite. By D. H. Tennent.

The experiments described were conducted for the purpose of determining the adult form and the life history of the trematode, of which *Bucephalus haimeanus* Lacaze Duthiers (*Bucephalus cucullus* McCrady), abundant in oysters of various regions, is the cercaria.

An account of the varying degrees of infection, from that of the presence, in the visceral mass, of a simple unbranched sporocyst, to the almost complete replacement of the gonads and liver by the long germ tubes, or branches of the sporocyst, was first given.

The first experiments described were conducted for the purpose of determining whether infection might spread directly from one oyster to another. Five experiments covering about one year, were mentioned.

In the second experiments conducted, an attempt was made to obtain the adult form by feeding infected oysters to fish which were kept in aquaria. These experiments seemed to show conclusively that the host of the adult form of *Bucephalus* was not necessarily an oyster-eating fish.

While these experiments were being carried on, a search for *Gasterostomum*, to which *Bucephalus*, by reason of its structure, would seem to be intimately related, was being made. The animals and plants found in the regions in which infected oysters occur, were thoroughly examined.

Gasterostomum was ultimately found in abundance in the garfish, *Tylosurus marinus.*

After determining the food of the gar, collections of the various fish and crustacea included in this list were examined.

In the viscera of the silverside, *Menidia notata*, specimens of which had been found in the stomach of the gar, encysted forms, little different in appearance from the cercariæ present in the oyster, were obtained.

The final set of experiments consisted in feeding viscera of the silverside to four species of fish, previous examinations having shown that in these species *Gasterostomum* does not occur.

These experiments showed that the encysted forms from the silverside were able to resist the action of the digestive juices of the fish to which they were fed and that they attached themselves to the wall of the intestine of their new host and continued in their development.

The evidence afforded by these experiments and observations showed that during its life history *Gasterostomum*, parasitic in the digestive tract of *Tylosurus marinus*, passes its cercaria stage

(*Bucephalus haimeanus*) in the oyster; that the cercariæ becoming mature leave the oyster and swim about in the water; that they are from thence obtained as food by the silverside and other small fish; that in these fish they encyst, finally to be released, and become sexually mature when their hosts are eaten by *Tylosurus*.

The extent of infection, the character of the regions in which infected oysters are found, the effect of the parasite upon the oyster, the origin of the germ cells from which the cercariæ arise and the significance of the differences in appearance and rate of growth in the experiment fish were also mentioned.

The experiments described were conducted at the Beaufort, N. C., Fisheries Laboratory.

July 19. **Relation of Ants to Temperature and Submergence.**

By ADELE M. FIELD.

An artificial nest was made, having as its basis a half-cylinder of copper, with ends projecting beyond the nest. One end of the copper was heated by an alcohol flame, while the other end was surrounded by ice. Different parts of the nest presented diverse temperatures, ranging from 10° C. or 14° F. to 60° C. or 140° F. Ants of various species were introduced into this nest, and it was found that the temperature preferred by them was from 24° C. or 76° F. to 27° C. or 82° F. Below 15° C. or 60° F. the ants become sluggish, and increase of temperature was accompained by increase of activity. Above 30° C. or 86° F. they manifested discomfort or distress. Exposure to heat so great as 50° C. or 122° F. for a period of fifteen seconds for the smaller ants, or of two minutes for the larger ants, invariably killed them, probably by coagulation of their protoplasm. The effect was the same whether the heat was applied through a wet or dry medium.

Ants of the same species as those killed by heat, survived submergence in cool water for seventy-two hours. The futility of ploughing up ant-nests with the expectation that the spring rains would destroy the ants was alluded to, and the application of heat was suggested as a better means of destroying these pests on farms.

July 22. **Habits and Life-History of Parasitic Copepods.**

By Charles B. Wilson.

A statement was first made of some of the problems confronting the student of this group, for only one of which has there been any attempt at solution. This is the one which logically comes first, the determination and description of species. But it is the least important and should be quickly followed by a careful study of habits, including relative abundance and the influence of the parasite upon its host. The life-history must also be worked out before adequate methods can be devised for exterminating the parasites or checking their ravages. Then come a series of ecological problems for which these copepods afford exceptionally fine material, color protection, mimicry, adaptation to environment, degeneration phenomena, and the like.

Material was shown for several of these problems, with a series of drawings for the life-history of the Caligidæ, the largest family in the group. The history of three members of this family, *Caligus rapax*, *Caligus bonito* and *Alebion glabrum*, was presented in full, that of others only partially.

The eggs hatch into a typical nauplius which swims about freely at the surface. After the second or third moult it becomes a metanauplius whose second antennæ cease to function as locomotor and become prehensile. At this stage it seeks its host and fastens to it.

In *Caligus*, *Lepeophtheirus* and allied genera an attachment filament is developed at the next moult from a median frontal gland which holds the larva securely to its host until it has matured. In *Alebion* and allied genera the second antennæ continue to serve as organs of attachment up to maturity. In the metanauplius there are but two pairs of thoracic appendages ; a pair is added at each successive moult up to the normal number.

These parasites do not usually injure their host, but may become sufficiently numerous to cause serious damage, especially if the host has been weakened or injured.

They feed upon the blood of their host which they obtain by piercing the skin in the vicinity of blood vessels and extracting the blood by means of a suctorial mouth tube.

They often become a serious nuisance to the artificial breeder of fish in ponds or aquaria. The only remedy in the case of the adults is to remove them carefully from the fish, but further trouble can be avoided by a plentiful introduction of small surface fish, sticklebacks, minnows, etc., which will eat up the larvæ of the parasitic copepods.

July 26. **The Nature of the Heart-Rhythm.** By A. J. CARLSON.

The following points were demonstrated on the heart of *Limulus.*

1. The heart-beat is neurogenic, not myogenic. The heart-muscle is not automatic under the normal conditions of life. Extirpation of the ganglion or nerve-cord on the dorsal side of the heart abolishes the rhythm at once and permanently, the muscle contracting only on artificial stimulation.

2. Coördination or conduction in the heart takes place in the nervous and not in the muscular tissue. The entire heart, save the median nerve-cord and the lateral nerves, may be cut transversely. This does not affect conduction or coördination. Severing the nerve-cord and the lateral nerves, leaving the heart-muscle intact, abolishes coördination of the ends of the heart at either side of the lesion, the contraction not passing the level of the lesion in either direction.

3. The inhibitory nerve-fibers act on the ganglion cells in the heart and not on the heart-muscle. Cardiac inhibition falls within the category of inhibition of automatic or reflex neural processes.

August 2. **Toxic and Antitoxic Action of Salts.** By ALBERT P. MATTHEWS.

August 6. **Causes of Blue and Green in Feathers.** By R. M. STRONG.

There are no blue pigments known in feathers, excepting one possible case cited by Häcker, and green feathers rarely owe their colors to green pigments. The blues are so-called structural colors confined to the barbs, usually.

Walter's hypothesis that these phenomena are *surface* colors produced by underlying melanin pigments is untenable because the pigments when isolated are not blue but dark brown.

There are serious objections to the turbid medium hypothesis of Häcker and others. Experimental studies indicate that most of the blue is produced, not in the horn substance of the feather, but at the dorsal inner surfaces of medullary cells where the incident light passes from the horn substance into the air filling the cavities of the medullary cells. The blue color is probably due to the stronger refraction of the blue end of the spectrum with consequent greater reflection of the blue.

The greens are usually produced by feathers which do not differ essentially from blue feathers except in having a yellow pigment in the cortex. The yellow pigment absorbs the incident blue, but transmits the green rays. The latter are reflected more strongly than the rays on the red side of the green in the spectrum, and they consequently give the feather its green color.

Many variations occur in the shape of blue and green barbs, and the latter very commonly have a high elevation of the dorsal cortex which contains a diffuse yellow pigment. The barbules are usually reduced or absent.

In some species, we find the green changing to dark dull yellow when the angle of incidence becomes very large. This phenomenon does not belong to the category of the common metallic or iridescent colors. It depends on purely mechanical conditions. The green disappears when the angle of incidence becomes so great that only the dorsal, yellow cortical elevations of the barbs are in view.

August 9. **Rheotropism in Fishes.** By E. P. Lyon.

It seemed impossible to the author that pressure could be the method of stimulation which brought about orientation of fishes in a current of water, according to the usual theories on the subject. The effect of the current would seem to be simply to carry organisms having approximately the same specific gravity as water down stream. No pressure would result unless the animal became oriented and swam against the current. It therefore seemed that points of reference on the bottom or banks must be as important in stimulating the fish as the current itself. Testing this hypothesis it was found that the fish responded to any movement of the bottom of the dish in which they were kept, turning in the same direction as the moving objects. By a series of experiments it was definitely made out that by far the largest element in securing orientation of the fishes experimented with is an optical reflex of such a kind that the animal tends to retain the same visual field. It is found however that blind fishes or fishes in the dark are able to orient themselves. Investigation of this phenomenon showed that it is primarily due to objects on the bottom. Blind fishes do not orient themselves in a uniform current unless they touch objects which are stationary. In more violent streams of water where there are considerable differences of velocity between closely proximated parts of the stream, orientation may occur. Here apparently the same explanation holds, *i. e.*, that the relatively stationary water constitutes the reference points by which the animal establishes its direction in the stream.

It is believed that pressure in a gross, mechanical way cannot explain rheotropism, but rather that it is always a response to the relative motion between the fish being carried passively down by the moving water, and more stationary parts of the environment.

August 12. **Osmotic Pressure of Sea Water and Marine Animals.** By W. E. Garrey.

1. The osmotic pressure of the sea water was determined by testing the depression of the freezing point (Δ) with a Beckmann apparatus.

(*a*) Buzzards Bay water, $\Delta = -1.83^\circ$ and -1.82°.

(*b*) Eel Pond water, $\Delta = -1.77^\circ$.

(*c*) Salt water tap of Marine Biological Laboratory showed considerable variations; $\Delta = -1.86^\circ$ in the early part of the season and as low as -1.78° after heavy rains. Most of the readings however varied between -1.84° and -1.82°.

(*d*) Basin of Fish Commission -1.84° and -1.81°.

These waters thus show a freezing point slightly less than that of molecular cane sugar ($\Delta = -1.85^\circ$) and the osmotic pressure is about 22 atmospheres calculated at 0° C.

2. The body fluids of marine invertebrates have an osmotic pressure very close to that of the sea water from which they are taken.

3. By immersion in diluted or concentrated sea water marine invertebrates take up or lose water respectively and assume an osmotic pressure approximating that of the external medium.

4. Selachians have an osmotic pressure equal to that of the sea water and change with the external medium, although not so readily or completely as invertebrates. They die if the change in osmotic pressure is great.

5. Marine teleosts have an osmotic pressure only about half that of the sea water; $\Delta = -0.80°$ and $-0.96°$. Transferring common eels from salt into fresh water did not lower the osmotic pressure of their blood. *Fundulus Heteroclitus* could also be placed in fresh water or doubly concentrated sea water and live for weeks. If, however, the body was partly scaled, or part of the skin removed, the fish die in normal sea water and in fresh water, but can be kept indefinitely in sea water which has been diluted with an equal volume of distilled water and which therefore has an osmotic pressure approximating that of their blood. These experiments point to the normal impermeability of the teleost integument.

August 12. **Coagulation of the Blood.** By LEO LOEB.

In former investigations I made it very probable that the so-called first coagulation of the blood of certain arthropods and the coagulation of the blood of *Limulus* consists in an agglutination of blood cells, that in the second coagulation of arthropods, on the other hand, fibrin is formed from fibrinogen under the influence of substances present in the blood cells and in the tissues. The latter, which I called tissue coagulins, are, within certain limits, specific, the former being not at all or much less specific. Similar conditions are present in vertebrate blood.

We have therefore to distinguish between two kinds of substances which may, under ordinary circumstances, affect the coagulation of the blood, namely, substances present in the serum and those present in the tissues (tissue coagulins).

Quite recently several investigators (Morawitz, Fuld u. Spiro) have advanced the theory that the tissue coagulins act only indirectly by transforming (to use the terms of Morawitz) thrombogen into prothrombin, which, by the aid of calcium, becomes the active ferment. This is a somewhat modified form of Alexander Schmidt's theory of the coagulation of the blood. Certain facts, however, made it appear to me more probable that the tissue coagulins attack the fibrinogen directly and transform it into fibrin. That this actually does take place I believe to have been able to show in the invertebrate blood (lobster), insofar as tissue coagulins still cause the coagulation of the blood plasma, after the substances present in the blood which accelerate the coagulation of the blood have been previously destroyed by heat.

It seems permissible to apply this result to vertebrate blood, the similarity between the factors causing the coagulation of vertebrate and invertebrate blood being very great.

In connection with the foregoing experiments a comparative study of the conditions under which these two substances act was made (influence of dilution, of calcium and other salts, power of resistance, preparation of active extracts). Their action on artificially prepared fibrinogen was also compared.

New facts were found which support the view formerly expressed that the coagulation of other arthropods represents an agglutination of the blood cells. In accordance with this view, I could prepare fibrinogen from lobster blood, in which a second coagulation takes place. On the other side, no fibrinogen could be prepared from *Limulus* blood, which has no second coagulation.

2. Botanical Lectures and Seminars in 1903.

July 8, Dr. George T. Moore : " The Polution of Public Water Supply by Algæ."

July 10, Dr. Bradley M. Davis : " The Relationships of the Sexual Organs of Plants."

July 20, Dr. Henry Kraemer : " The Unorganized Contents of the Plant Cell."

July 21, Dr. Henry Kraemer : " The Structure of the Cell Wall."

July 27, Mr. Herbert J. Webber : " History and General Factors of Plant Breeding."

July 28, Mr. Herbert J. Webber : " The Principles of Selection and Isolation in Improving Breeds."

July 29, Mr. Herbert J. Webber : " The Mutation Theory of De Vries."

July 30, Mr. Herbert J. Webber : " The General Laws of Hybrids with a Discussion of Mendel's Principles."

July 31. Mr. Herbert J. Webber: "The Utilization of Hybrids in Practical Plant Breeding and the Selection of Vegetative Parts in Breeding."

August 5, Dr. Bradley M. Davis: "Cytoplasmic Structures of the Plant Cell."

August 6, Dr. Rodney H. True: "Theory of the Nature of Solutions. Dissociation Hypothesis and Objections to it."

August 7, Dr. Rodney H. True: "Toxic Action of Solutions on Plants, caused by Solutions Containing One Solute."

August 10, Dr. Rodney H. True: "Toxic Action of Solutions on Plants Caused by Solutions containing more than one Solute."

August 11, Dr. Rodney H. True: "Influence of Solutions on Plant Functions and Structure."

August 11, Mr. Christopher S. Oglevee: "The Influence of Insoluble Substances on the Action of Poisons in Solutions."

August 12, Dr. Rodney H. True: "Artificial Sea Water."

July 8. The Pollution of Public Water Supplies by Algæ.

By George T. Moore.

The importance of a scientific investigation of this most common cause of the bad odors and tastes in drinking water was pointed out and numerous examples were given of the serious inconvenience and great financial loss due to the presence of algæ in reservoirs. It is necessary to recognize that the means of finding a remedy for the trouble must be biological rather than chemical or mechanical, and it is only after we are familiar with the life history of the organisms producing the odor and taste that we can hope to find a remedy.

Certain experiments then under way, being carried on by the Department of Agriculture, were described, and it was believed that the result of these trials would prove the discovery of a cheap and practical remedy which could be used on the largest scale and yet not cause trouble to the consumer.

July 10. The Relationships of the Sexual Organs of Plants.

By Bradley Moore Davis.

The types of sexual organs found in plants, their structure, origin and relationships were considered. These types fall into three classes: (1) the unicellular sexual organs (gametocysts) (2) the multicellular sexual organs (gametangia) and (3) the peculiar multinucleate gametocysts which become sexual cells (cœnogametes). The first class (gametocysts) is found throughout the Thallophytes, and the evolutionary principles governing their differentiation into spermatocysts and oöcysts are well understood. The second class (gametangia) is characteristic of the bryophytes and pteridophytes and contains the well-known antheridium and archegonium of these groups. The possible origin of these structures from the plurilocular sporangium, according to the writer's recently published hypothesis, was discussed. The third class (cœnogametes) comprises the remarkable cœnocytic sexual cells found among the Mucorales, Saprolegniales and Peronosporales. Great interest attaches to their behavior and phylogenetic relations on account of the peculiarities of their structure, which are not found in any other group of organisms. The author's explanation of these conditions was presented in the light of his recent investigations on oögenesis in *Vaucheria*.

July 20. **The Unorganized Contents of the Plant Cell.** By HENRY KRAEMER.

The micro-chemical and micro-physical properties of the following unorganized cell contents were considered: (1) Product of constructive metabolism; the crystalloidal carbohydrate, starch. (2) Products of reconstructive or destructive metabolism; *A*, organic substances — (*a*) occurring in the protoplasm and cell-sap, as oils, resins, ferments and proteine crystalloids; (*b*) occurring in the cell-sap, as crystalline carbohydrates (dextrose, maltose, sucrose, etc.) and the crystalloidal carbohydrate inulin; (*c*) occurring in cell-sap or cell-wall, colloidal carbohydrates (gums, mucilages and pectins), tannin, alkaloids, glucosides and calcium oxalate; *B*, inorganic substances — (*a*) calcium carbonate in cystoliths or in the cell-wall; (*b*) silica in irregular masses or in the cell-wall.

July 21. **The Structure of the Cell-wall.** By HENRY KRAEMER.

The various methods for differentiating the different lamellæ of the cell-wall were first considered. The similarity in the structure of the starch grain and that of the cell-wall was demonstrated by micro-physical means, the use of iodine solutions and aniline stains. The various types of cell-walls and modified cell-walls were described as follows: (1) Cellulose walls; (2) ligno-cellulose walls; (3) adipo-cellulose walls; (4) mucilage-cellulose walls; (5) pecto-cellulose walls; (6) reserve-cellulose walls. In connection with the study of the reserve-cellulose walls the work of the author on the continuity of protoplasm was considered, and the similarity of the structure of the walls of this class to the structure of the wheat starch grain was pointed out.

July 27. History and General Factors of Plant Breeding.

By HERBERT J. WEBBER.

This lecture included a discussion of the history of breeding from the time of Fairchild who made the first plant hybrid in 1719 to the present time. The principles of breeding advocated by Knight and Van Mons were compared with later methods and the gradual improvement traced.

Illustrations were given of physiological variations, individual or congenital variations and mutations or saltatory variations, showing how the two latter forms of variations have been utilized by practical breeders, while physiological variations have proven valueless because not hereditary.

The factors of breeding, including the general laws of heredity, transmitting power, unity of individual isolation and selection, were considered briefly.

The speaker emphasized the importance of breeding for a special purpose and with a definite idea in view. The breeder should be familiar with all varieties, races, and species of the plant which he proposes to improve, and select as the parent or parents for his new sort, the existing variety or varieties which exhibit in the greatest perfection the ideal characters which he desires to combine.

In the amelioration of a wild species it has been claimed since the time of Knight that it is first necessary to "break the type" as it is expressed, to get the plant into a condition of variation. It is claimed that a wild plant is for some time very stable and but slightly variable under conditions of cultivation, and that by continuous high cultivation this stability can be broken and the plant changed into a condition of variability, a condition which would appear comparable to the mutation period predicated by De Vries. The evidence in favor of the theory of breaking the type is slight being based mainly on the experiments of Knight and H. Vilmorin.

July 28. The Principles of Selection and Isolation in Improving Varieties. By HERBERT J. WEBBER.

This lecture described in detail the methods of selection used in originating new races and strains of wheat, corn, cotton, sugar, beets, and other agricultural crops. The nursery method of planting introduced by Hallett and used extensively in this country by Professor Hays and others was compared with the field method of selection introduced by Rimpau, and illustrations were given of the use of each method in the production of new forms. The nursery method gives to each plant all the space it requires and allows the plant to show what it will do under the most favorable conditions. The field method provides for the growing of the plants under the conditions of field culture where different individuals compete with each other in a struggle for existence, the same as occurs in the ordinary conditions of culture under which the plant is grown.

The importance of considering the individual as the unit of selection under ordinary conditions was emphasized but it was pointed out that in some cases the selection of a fraction of an individual will give quicker results. In corn for instance, the kernels on an ear may show several different colors and it was demonstrated that a uniform color can be secured more quickly by separating out these kernels which show the desired color. In hybrids of smooth and fuzzy-seeded cottons on the same plant, some bolls may have nearly smooth seeds, while others have fuzzy seeds in various degrees. It has been found that the seeds in a single boll run very uniform either smooth or fuzzy, and the evidence obtained on this point indicates that a larger percentage of individuals producing smooth seed can be obtained by selecting seed only from bolls producing smooth seed. These points were emphasized to show that breeders must be prepared to take advantage of every important point that may appear.

July 29. **The Mutation Theory of De Vries.** By HERBERT J. WEBBER.

The speaker called attention to the fact that very many of the races and varieties of our cultivated plants have originated as sudden variations. Individual variations were discussed in comparison with mutations and it was claimed that no strict line of demarkation can be drawn between the two. The work of selections deals mainly with slight individual variations, some of which as shown by experiment have strong transmitting power and reproduce themselves true to type in large degree. No means exists of distinguishing between these and small mutations which are mainly transmitted true through the seed. The difference is one of degree only, it was claimed, and cannot be detected with certainity.

The influence of natural selection in the origin of natural species is not eliminated by accepting the idea that variations that form species are produced as mutations. Mutations of all kinds are doubtless formed, desirable and undesirable, fit and unfit, and only those maintain themselves and form permanent species that are fully in harmony with the environment and thus survive. Undesirable mutations are weeded out by natural selection. Even granting the occurrence of a mutation strikingly different from the parent type and thoroughly fitted to the environment, something more is necessary other than natural selection to insure its forming a new species. It is of primary importance that the mutation or variation have strong transmitting power, giving progeny like the parent mutation. Aside from this, some form of isolation is necessary to secure the formation of the new type as the few plants showing the variation would be swamped by panmixia. This swamping the speaker pointed out could be overcome or avoided in at least three ways ; namely, (1) geographical isolation, (2) tendency to self-fertility, or prepotency of pollen, (3) a tendency to prepotency or preponderance of type. The first of these, the influence of geographical isolation, has been emphasized by Gulich and Romanes, and its general application is familiar to all through the classical illustration of snails in different valleys in the Sandwich Islands given by Gulich. The influence of the other two factors the speaker has never seen emphasized although he is inclined to believe that they are very important factors in evolution. The second factor which is a form of isolation, namely tendency to self-fertility or prepotency of pollen, would be present whenever the flowers of the plant are modified to insure self-fertilization, as in the case of the pea, bean, and many other legumes, in wheat, barley, and some other plants, and in cases which may occasionally occur where the mutation gives rise to a plant which has a strong tendency towards self-fertility, the progeny of cross fertilization with pollen of another individual being fewer in number and lacking in vigor. Such a mutation was observed and experimented with by Darwin and graphically described under the caption "The Hero Morninglory."[1] The races of Cupid sweet peas, and various wheat races are illustrations of cultivated species, we may call them, that have originated in this way. Given a mutation suited to the environment, having strong transmitting power, and a tendency to self-fertility, or some device insuring self-fertility and we have the creation of a new species.

The third means by which the swamping effect of panmixia may be overcome is where a tendency to prepotency or preponderance of type is formed. In such instances the mutation, giving rise to a type which while not necessarily preferring self-fertilization is strongly prepotent and dominant in its action and transmits its characters to all its progeny which bred *inter se*, would probably strengthen the type and lead to its gaining a permanent foothold. Such illustrations, like the Ancon or Otter sheep are not uncommon among domestic animals and plants.

July 30. **The General Laws of Hybrids with a Discussion of Mendel's Principles.** By HERBERT J. WEBBER.

The claim advanced by Mendel and some of his followers that of a certain allelomorph or character pair, one character is dominant and masks the other character in first generation hybrids was claimed by the speaker to have but limited application. Many cases of blends of two characters were cited. It was also pointed out that the statement that first generation hybrids are of the same type is erroneous. The case of hybrids of orange

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[1] Darwin, Cross and Self-fertilization in the Vegetable Kingdom," p 47.

(*C. aurantium*), trifoliate orange (*C. trifoliata*), where each seedling differs from every other seedling, was cited.

It was shown that the conception of purity of the germ cell which is the primary feature of Mendel's laws must certainly be modified as the fact of reversions to ancestral characters in types that have bred true for generations show that the "Analage" of the character must have remained in the germ cell through all these generations without exerting or showing itself. Cases of reversion in cotton and other plants were cited as illustrations.

The principle of the segregation of characters in the formation of the germ cells was also denied from the fact that many hybrids have been bred into fixed races coming true to seed that exhibit a heterozygote character intermediate between the two parental characters. Such races are the white cap dent corn, a hybrid of a yellow and a white dent which is yellow on the sides and white on the apex of the kernel; the Griffin, Allen hybrid, and Doughty cottons which are hybrids of sea island and upland cotton that have fibers intermediate between the two parents in length and fineness.

Mendel's researches were considered by the speaker to be of the utmost importance from the theoretical scientific standpoint, but it was claimed that they will have little or no effect in changing the methods of practical breeding.

July 31. The Utilization of Hybrids in Practical Plant Breeding; and the Selection of Vegetative Parts in Breeding. By Herbert J. Webber.

While pure selection gives the quickest and surest results in breeding, it is to hybridization that we must give attention when we desire to produce striking new sorts or combinations of the good qualities of two or more existing sorts with the elimination of the undesirable features. It is very necessary to carefully select the races or species to be combined, and also just as necessary to select the best individuals of these races or species that are to be used as the parents.

The second generation is the variable hybrid generation from which our selection of desirable combinations must be made. This is in accordance with Mendel's conclusions but was known and used in practice long before the rediscovery of Mendel's works.

The fixation of select hybrids into stable races is best accomplished by isolating the progeny of the select plant and breeding together those individuals of the progeny which are nearest alike. Probably fixity or uniformity could be gained quicker by in and in breeding, but in plants that are normally cross fertilized the loss of vigor and fertility from the inbreeding would probably in most cases render the use of this method impractical.

Attention was directed to the important improvements that can be secured by the selection of vegetative parts. By the selection of vigorous growing cuttings of the carnation, violet, rose, etc., the size and number of the flowers produced can be greatly increased. Many bud sports or bud mutations have been used as new varieties and are valuable acquisitions.

Plant breeding, it was pointed out, is not necessarily a difficult task. The speaker urged the great importance of the work and the necessity of more extensive scientific investigations and practical experiments.

August 5. **Cytoplasmic Structures of the Plant Cell.** By BRADLEY MOORE DAVIS.

The writer described in this seminar the principal structures and activities of the cytoplasm in different parts of the plant cell and at different periods of ontogeny. There are three principal regions of the cytoplasm: (1) The plasma membranes (kinoplasmic in character), which surround the protoplast, the vacuoles and the nucleus, (2) trophoplasm, and (3) the kinoplasm intimately concerned with mitotic phenomena and which finds morphological expression in asters, centrospheres, centrosomes and filarplasm. The structure of trophoplasm was described together with the peculiar physodes, nematoplasts and cœnocentra. But the main discussion concerned the behavior of kinoplasm during mitosis and the segmentation of the protoplasm at cell-division. Many of the kinoplasmic structures characteristic of these events may be closely related to one another, in spite of their diverse morphology, when studied ontogenetically, and the author discussed some of these problems with especial reference to his studies on the Hepaticæ and Thallophytes.

August 6. **Theory of the Nature of Solutions. Dissociation Hypothesis and Objections to It.** By RODNEY H. TRUE.

August 7. **Toxic Action of Solutions on Plants Caused by Solutions Containing One Solute.** By RODNEY H. TRUE.

August 10. **Toxic Action of Solutions on Plants Caused by Solutions Containing More than One Solute.** By RODNEY H. TRUE.

August 11. **Influence of Solutions on Plant Functions and Structure.** By RODNEY H. TRUE.

These seminars presented a discussion of the theory of electrolytic association and its bearing on plant physiology. The theory was outlined and some of the more recent objections to it were stated, together with some of the more important grounds on which such objections are based. Attention was called to the bearing of these objections, with reference to physiological research along these lines, and caution was advised in construing results in terms of the theory. It was pointed out that, as long as a considerable mass of evidence lies against this hypothesis, physiologists should not commit themselves too thoroughly to the theory and its conclusions. The toxic action of various classes of compounds was then discussed in the light of the dissociation hypothesis and the relation between physiological action and molecular structure was pointed out. Some relations obtaining between the structure and functions of plants and the action of molecules and ions was discussed.

August 11. **The Influence of Insoluble Substances on the Action of Poisons in Solution.** By CHRISTOPHER S. OGLEVEE.

Seedlings of *Lupinus albus* were grown in beakers containing the various poisons. The concentration which would kill the radicle was determined. It was found that finely divided particles of an insoluble substance placed in the beaker containing the poisonous solution not only allowed the radicle to grow, but often accelerated the growth above the normal, and produced effects similar to those of a more dilute solution. The insoluble substances used, sand, pounded Bohemian glass, shredded filter paper, potato starch, and paraffine, were cleaned and washed as thoroughly as possible. Some of the poisons used were copper sulphate, silver nitrate, mercuric chloride, citric acid, resorcin, phenol, thymol, etc. The effects could not be due to the action of the insoluble particles on the ions of the poisons which do not dissociate. The variety of insoluble substances used would indicate that the effects were not due to the slight solubility which some of the particles possess. Since no othei suggestion presents itself, perhaps the theory of " adsorption " offers the best explanation to the problem.

August 12. **Artificial Sea Water.** By RODNEY H. TRUE.

The experiments now in progress at the United States Fish Commission in connection with the attempts to make artificial sea water capable of sustaining marine life were discussed. Two sorts of solutions were compounded, one a synthetic, prepared by adding to distilled water the required salts in quantities indicated by analysis. The second solution was obtained by dissolving in distilled water a complete sea salt obtained by evaporating sea water to dryness over steam. The importance of the presence of the full amount of calcium was pointed out and the utility of charging the solutions with carbon dioxide gas was noted. The report of the results thus far obtained with the marine plants and animals indicated that the synthetic solutions are less satisfactory than those obtained from evaporated sea salt. The hope was expressed that the latter might be to a considerable degree satisfactory for the demands in this direction, but the fact that experiments thus far have covered only two summer sessions was cited as ground for a cautious interpretation of these results.

3. BOTANICAL LECTURES AND SEMINARS IN 1904.

July 12, Dr. George T. Moore : " Botanical Work at Several Botanical Laboratories in Holland, Scandinavia and Russia."

July 13, Dr. George T. Moore : " The Physiological Methods of Purifying Water Supplies."

July 15, Mr. Mintin A. Chrysler : " Regeneration in Plants as Illustrated by Zamia Floridana."

July 19, Miss Etoile B. Simons : " Morphological Studies on Sargassum Filipendula."

July 22, Dr. James J. Wolfe: "Cytological Studies on Nemalion."

July 27, Dr. Henry Kraemer: "The Origin and Nature of Color in Plants."

August 1, Dr. G. F. Blakeslee: "Sexual Reproduction of the Mucorineæ."

August 10, Dr. Bradley M. Davis: "The Algal Flora of the Bay of Naples."

July 12. **Botanical Work in Several Botanical Laboratories of Holland, Scandinavia and Russia.** By DR. GEORGE T. MOORE.

A popular account of a recent visit made to some of the principle botanical laboratories in the countries mentioned. A description of the facilities for work at Delft, Amsterdam, Copenhagen, Christiania, Bergen and Stockholm, with a brief reference to the principle botanists in these cities was given, as well as a more detailed account of the Institute for Experimental Medicine at St. Petersburgh and its illustrious director, Winogradsky.

July 13. **The Physiological Method of Purifying Water Reservoirs.** By DR. GEORGE T. MOORE.

After a brief reference to the very widespread difficulty in water supplies due to algæ, and the failure to devise any means of removing or preventing the bad odors and tastes, an account of the toxic effect of copper sulfate upon algæ was given. It has been known for a long time that many of the heavy metals were extremely poisonous to some plants, and experiments showed that copper sulfate could be used at a dilution so high as to be absolutely harmless to man and yet sufficient to destroy or prevent the growth of algal pests. Practical application of the method to reservoirs containing millions of gallons has shown that the necessary requirements of efficiency, cheapness and harmlessness to man are all complied with and it is believed that a thoroughly reliable means has been devised for preventing the growth of algæ in water supplies.

While the application of the method to the destruction of typhoid and cholera is not universal as with the algæ, it is believed that under certain conditions the use of copper sulphate offers the only known means of thoroughly and quickly sterilizing a reservoir.

Extensive experiments on a large scale are now being carried on and results already obtained warrant the conclusion that the method is satisfactory in every respect.

July 15. **Regeneration in Plants as Illustrated by Zamia floridana.** By Mr. Mintin A. Chrysler.

Zamia floridana exhibits a remarkable capacity for producing new shoots and roots from pieces of stem. A piece no larger than a walnut can give rise to a new plant. The new shoots proceed in most cases from the vascular region of the central cylinder, but may arise from the neighborhood of the periderm, or may form a cap over the whole central cylinder. Only the last case merits the term "regeneration"; the others are merely instances of adventitious budding. Roots may spring from the cut surface just as do the shoots. The power of producing new shoots seems to be shared by all tissues which have remained meristematic, or which have resumed the power of cell division such as the phellogen of the wound cork. A case of budding from the hypocotyl has been observed. The theories of "latent buds" and "polarity" are not supported by the specimens of *Zamia* examined.

July 19. **Morphological Studies on Sargassum Filipendula.** By Miss Etoile B. Simons.

The conceptacle, antheridium, oögonium, and cryptostoma were discussed in detail.

Sargassum Filipendula is unlike the accounts of authors who have studied various other members of the Fucales in the development of its conceptacle.

Bower in his article "On the Development of the Conceptacle in the Fucaceæ" describes a single superficial initial cell, which usually disintegrates. According to his account cells adjacent to this initial divide and form the conceptacle. Holtz announces a group of initial cells in *Pelvetia fastigiata* which disintegrate. Below these and other disintegrating cells are found the elements which form the conceptacle walls.

The conceptacle of *Sargassum* originates with a single superficial cell. Cell division beginning in this initial continues in the progeny until the conceptacle is formed. The entire organ, therefore, is the descendant of one cell, the true initial.

The antheridium and oögonium develop from wall cells of the conceptacle as in other members of this group. The oögonium has no pedicel, however, and usually contains but one egg.

The cryptostoma develops from a single superficial cell as does the conceptacle and is homologous with that organ.

July 22. **Cytological Studies on Nemalion.** By Dr. James J. Wolfe.

The chromatophore of *Nemalion* has a hollow ellipsoid form from which processes radiate to the periphery of the cell and there flatten out to form a clathrate membrane. The region surrounded by the ellipsoid portion of the chromatophore, and generally regarded as a pyrenoid, consists entirely of vacuolar material.

The sex-organs cannot be regarded as unicellular structures; since in earlier stages the trichogyne possesses a well organized nucleus, which fragments as that organ matures; the egg-cell thus becoming an intercalary cell, and the trichogyne one which has been specialized in connection with the reproductive processes.

The nucleus of the spermatium normally divides into two fertilizing elements which are discharged into the trichogyne; which events show the so-called spermatium to be an antheridium.

In the mature cystocarp the ultimate cells give rise terminally, as well as by subterminal proliferation, to a variable number of carpospores, which is further augmented by repeated proliferation within the successively formed mother-cell walls.

The entire chromatin content of the nucleus is stored in the nucleolus, and in the prophases of division passes to the nuclear wall along delicate fibrillæ. The spindle is intranuclear, and centrosomes are distinctly visible at metaphase.

The conclusion that *Nemalion* presents the essentials of an antithetic alternation of generations, and that the cystocarp is, therefore, the homologue of the sphorophyte in higher plants, is indicated by cytological evidence — since approximately sixteen chromosomes are present in the divisions of the cells of the cystocarp up to the period of spore formation, and approximately eight in those of the thallus, the reduction division being immediately associated with the production of carpospores.

July 27. **The Origin and Nature of Color in Plants.** By Dr. Henry Kraemer.

Color in plants is due to definite constituents which either themselves are colored or they produce colors when acted upon by other substances. These constituents are either associated with some of the organized bodies of the plant cells, or they occur in the cell-sap. To the former belong the pigments associated with the etioplasts, chloroplasts and chromoplasts. These are distinguished from all other color substances of the plant by their solubility in ether, benzol, xylol, chloroform and similar solvents.

Besides the plasted pigments there are substances dissolved in the cell-sap. These occur in all parts of the plant and give rise to the other shades and tints than yellow and green. They are quite soluble, usually in a 50 per cent. hydro-alcoholic solution, and are insoluble in the above-mentioned solvents. They give well-marked reactions with certain groups of reagents and show many properties in common whether obtained from flowers, leaves, fruits, roots or stems.

The relationship of the chloroplastid to the production of starch has already been pointed out. The occurrence of proteid substances in chromoplastids suggests that they have the special function in the manufacturing or storing of nitrogenous food material which is subsequently utilized in the development of the ovule, germinating plant or biennial plant. The wide distribution of cell-sap colors, which share many general properties in common, suggests that these substances, like other unorganized cell contents, are but incidental to physiological activity.

August 1. **Sexual Reproduction of the Mucorineæ.** By A. F. Blakeslee.

According to their methods of zygospore formation the Mucorineæ may be divided into two groups. In the homothallic group zygospores are developed from branches of the same thallus. In the heterothallic group zygospores are developed from branches which necessarily belong to thalli diverse in character. Every heterothallic species is an aggregate of two distinct strains through the interaction of which zygospore production is brought about. These sexual strains in an individual species show a more or less marked differentiation in vegetative luxuriance which may be designated by (+) and (−) signs respectively. A process of imperfect hybridization will occur between unlike strains of different species. By taking advantage of this character it has been possible to group together in two opposite series the strains of all the heterothallic species under cultivation. When thus grouped the (−) strains will be in one series while the (+) will be in the other. In the two series are represented the two sexes.

August 8. **The Algal Flora of the Gulf of Naples.** By Dr. Bradley M. Davis.

This seminar considered the character and distribution of the marine algæ in the vicinity of Naples and was illustrated by a collection. Comparisons were made with the flora of the Atlantic coast of America south of Cape Cod which has some important features in common with the Mediterranean. The opportunities for botanical work in the zoölogical station at Naples were described.

THE OSMOTIC PRESSURE OF SEA WATER AND OF THE BLOOD OF MARINE ANIMALS.

Including Some Observations on the Permeability of Animal Membranes.[1]

WALTER E. GARREY.

Experimental work on the relation of salts and other substances in solution to the life processes of marine animals requires an accurate knowledge of the osmotic pressure of both the sea water and the body fluids of the animals. This knowledge should be supplemented by definite information relative to the permeability of the membranes of the animals under investigation. Thus far this field has been neglected by American investigators. It has been assumed that local conditions are similar to those existing along the European sea board. Owing to the character of the work in our Marine Laboratories it seemed advisable to make some observations which would place our knowledge of local conditions on a firmer basis.

Method.

Until recently our knowledge of sea water and animal fluids has been based solely on quantitative chemical analyses. F. Bottazzi[1] at Naples in 1897 calculated the osmotic pressure from the depression of the freezing point. This method has since been used by several investigators working in the same field (Rodier, Quinton, Frédéricq) and was adopted as most convenient for our purpose. Employing the Beckmann apparatus the freezing point is determined. For aqueous solutions a freezing point below that of distilled water signifies the presence of some substance in solution which is exerting an osmotic pressure. This pressure calculated for 0° C. is equal to about twelve atmospheres for a depression of one degree centigrade (— 1° C.). The depression of the freezing point is designated Δ. Owing to the super-cooling which takes place in solutions as strong as those with which we are dealing, inconstant results are obtained unless freezing is induced by innoculation with a tiny crystal of ice as soon as supercooling of about three tenths of a degree has taken place. A few earlier experiments in which this technique was neglected have not been recorded here. Invertebrate blood clots slowly and the first clot is easily broken up so that the freezing point of fluid as a whole may be determined. Teleost blood was whipped before freezing but no attempt was made to remove the corpuscles in as much as it has been found that they exert no appreciable effect upon the freezing point (Hamburger[11], Hedin[12]).

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[1] These investigations were made at the Marine Biological Laboratory, Woods Holl, Mass. They were reported to the Biological Seminar, August 12, 1904.[10] Investigations conducted on the Pacific Coast will be reported later as they are still in progress.

OSMOTIC PRESSURE OF SEA WATER.

The animals worked with at Woods Hole were obtained from so many different localities that it seemed advisable to determine the freezing point of the water from the same sources. The results of these determinations are given in Table I.

TABLE I.

	Buzzard's Bay.	Basin of the U. S. Fish Com.	Laboratory Tap.	"Eel Pond."
Maximum Δ	−1.835°	−1.84°	−1.84°	−1.82°
Minimum Δ	−1.81	−1.805	−1.82	−1.75
Average Δ	−1.818	−1.82	−1.82	−1.76
	Eleven samples.	Twenty-three samples.	Forty samples.	Eight samples.

The slight variations noted in the concentration of the different samples of sea water are not due to errors in observation for each sample of sea water was tested repeatedly and the results were checked by the use of three thermometers. The variations may be explained by the more or less land-locked condition of the bodies of water, the concentration being continually, though slightly, altered by the tides and by the continued advent of fresh water. After one extremely heavy rain the water of the laboratory tap showed considerable dilution Δ being — 1.78°. "Eel Pond" water also was diluted till the freezing point was only — 1.70°.

A glance at Table I. shows that the freezing point of the sea water at Woods Hole is on an average of 1.82° C. below zero. The osmotic pressure at a temperature of 0° C. is therefore about 22 atmospheres.

It is a noteworthy fact that the water at Woods Hole is more dilute than is that at Naples (Bottazzi[1]) where $\Delta = -2.29°$. At Arcachon also the sea water is more concentrated, Rodier[21] having determined its Δ to be − 1.89° C. According to our determinations made at Pacific Grove, California, the sea water of the Pacific Ocean freezes at − 1.90° C.

THE OSMOTIC PRESSURE OF THE BLOOD OF MARINE ANIMALS.

Invertebrates. — The body fluids (or blood) of a number of invertebrates were tested and in every case the freezing point was found to be the same as that of the sea water from which the animal was taken, no variation of more than two hundredths (0.02° C.) of a degree being found. The following list includes the forms worked with and indicates the fluid tested.

Echinodermata:

1. *Thyone briareus* — fluid from the perivisceral cavity.
2. *Arabacia punctulata* — fluid from the perivisceral cavity.
3. *Asterias vulgaris* — fluid from the perivisceral cavity.

Mollusca:

4. *Sycotypus canaliculatus* — blood obtained by section of the foot.
5. *Venus mercenaria* — blood obtained by section of the foot.
6. *Mya arenaria* — blood obtained by section of the foot.

Arthropoda:

7. *Homarus Americanus* — blood.
8. *Limulus polyphemus* — blood.

Selachians. — Two forms were worked with, the blood being obtained from the caudal artery.

TABLE II.

	Maximum Δ.	Minimum Δ.
1. *Mustelus canis*	− 1.90°	− 1.86°
2. Sand shark	2.03	− 1.88

The osmotic pressure of the blood and body fluids of *invertebrates* is due exclusively to the salts which are in solution, the proteid molecules being so large that they exert no appreciable osmotic pressure. Analyses made by L. Frédéricq[7] show that the salts in the blood of a large number of invertebrates are present in the same concentration as in the sea water. Although the blood of *selachians* has a freezing point approximately the same as that of the sea water, the salts are present in much smaller amount, 1.6 per cent. to 2.3 per cent. according to different analyses. The high osmotic pressure is maintained by the presence of a large and variable amount of urea (2–3 per cent., V. Schroeder,[21] Quinton,[20] Rodier,[22] Frédéricq[9]).

Teleosts. — The blood of all teleosts examined showed a low osmotic pressure which, in round numbers, approximated one half that of the sea water.* In Table III. are given the extreme variations in the freezing point for individuals of each species.

TABLE III.

	Source of Blood.	Maximum Δ.	Minimum Δ.
1. Sword fish	Heart after death.	— 0.96°	— 0.90°
2. Tautog. (*Tautoga onitis*)	Branchial artery.	— 0.86	— 0.86
3. Squeteague (*Cynoscion regalis*)	" "	— 0.864	— 0.86
4. Conger eel	" "	— 0.82	— 0.80
5. Common eel (*Anguilla chrysypa*)	" "	— 0.90	— 0.90

The results of all these investigations on marine animals agree with those of F. Bottazzi[1] and of Frédéricq[9] at Naples. These investigators found that invertebrate blood froze at $-2.03°$ as did also that of selachians, while teleost blood showed $\Delta = -1.04°$. The slightly greater depression of the freezing point found by these authors is to be accounted for by a greater concentration of the sea water at Naples than at Woods Hole.

VARIATIONS IN OSMOTIC PRESSURE OF THE BLOOD DUE TO CHANGES IN THE CONCENTRATION ON THE EXTERNAL MEDIUM.

The analyses of L. Frédéricq[7] showed that the concentration of the salts in the blood of invertebrates varied with the concentration of the salt water from which the animals were taken. This was most strikingly shown by the blood of *Carcinus mænus* taken from brackish water and from sea water. In the course of our investigations it was found that differences in the freezing point of the blood of invertebrates accompanied differences in the freezing point of the sea water from which they were taken; thus the blood of lobsters taken from the "basin" traps showed $\Delta = - 1.82^{\circ}$, but when taken from the "eel pond" $\Delta = - 1.77^{\circ}$. A decrease in the concentration of the water from the laboratory tap, due to severe rains, caused exactly the same changes in the freezing point of lobster's blood, Δ became $- 1.78^{\circ}$. An increase in the osmotic pressure of the blood of *Limulus* was induced by a two days' exposure to the drying influence of the atmosphere. The freezing point went down to $- 1.90^{\circ}$. The blood of a *Limulus* kept alive for two weeks in a damp cellar froze at $- 2.03^{\circ}$, the normal $\Delta = - 1.82^{\circ}$. Both diurnal and seasonal changes occur in the concentration of the water of San Francisco Bay, Cal. (taken near the Golden Gate), and the perivisceral fluid of starfish shows exactly the same changes; thus on March 17, 1904, $\Delta = - 1.47^{\circ}$ at high tide but at low tide only $- 1.385^{\circ}$. On September 23, $\Delta = - 1.80^{\circ}$.

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* Incidentally it was observed that the red corpuscles of teleosts were crenated by sea water.

With these facts as a starting point it was decided to test the freezing point of the blood when the animal was subjected to a large decrease or increase in the osmotic pressure of the external medium.

Dilution of the sea water was first tried and after a longer or a shorter immersion the animal was removed and the freezing point of the blood was determined. The changes which are thus induced are given in Table IV.

In nearly every experiment the animals were kept in the dilute medium until collapse set in, but in a majority of cases they were able to revive when replaced in normal sea water. *Limulus* and *Sycotypus* are particularly hardy and it is noted that the freezing point of their blood changes very quickly until in some cases it is approximately equal to that of the external medium. When the external medium is very dilute death may occur before this equalization takes place; this was particularly true in the case of *Homarus*, which is very susceptible to a change in the con-

TABLE IV.

	Sea Water Diluted with an Equal Volume of Distilled Water, $\Delta = 1.02°$.		Fresh Water, $\Delta = -0.02°$.	
	Duration of Immersion.	Blood Δ.	Duration of Immersion.	Blood Δ.
1. *Asterias*	7 hours.	$-1.14°$	12 hours.	much swollen.
2. *Sycotypus*	30 hours.	-1.07	12 hours.	$-1.23°$
			48 hours.	-0.67
3. *Nereis*	6 hours.	swollen.	3 hours.	swollen.
4. *Chætopterus*	6 hours.	swollen.	3 hours.	swollen.
5. *Limulus*	2.5 hours.	-1.43	8 hours.	-1.33
	52 hours.	-1.12	16 hours.	$-.90$
6. *Homarus*	2.5 hours.	-1.43	1 hour.	-1.63
	6 hrs. (dead).	-1.32		

centration of the external medium. In these experiments with dilute solutions it was found that when *Venus* was placed in fresh water it closed up so tightly that after two weeks' immersion the osmotic pressure of the blood was not lowered; perforation of the edges of the shell however admitted the fresh water and the osmotic pressure was lowered. A lobster placed in 3 vol. sea water + 1 vol. distilled water showed a lowering of the osmotic pressure, as indicated by $\Delta = -1.46°$. Marine flat worms on the bodies of Limuli became much swollen in the diluted sea water although they remain active for a long time.

Concentrated sea water obtained by evaporating until $\Delta = -3.80°$ was next used as the medium of immersion. The blood of *Limulus* after sixteen hours in this medium froze at $-3.79°$. The blood of *Homarus* in this doubly concentrated sea water froze at $-3.60°$ at the end of eight hours. In sea water concentrated to 0.8 its original volume the blood of *Homarus* froze at $-2.17°$ after two hours' immersion. These animals tolerate an increased osmotic pressure much better than the equivalent decrease. When the aquarium water is concentrated, complete equalization of enormous differences in the osmotic pressure between the external and internal media may take place without any marked symptoms of asthenia such as are seen when the external medium is dilute.

From the facts just sketched we see that all of the marine invertebrates which we have worked with are truly "poikilosmotic." Two factors may be at work in producing the variations in the osmotic pressure, viz., the interchange of water, and of salts.

The entrance of water is proven by the enormous increase in weight, and the swollen appearance of those animals which have been placed in diluted sea water. This swelling has already been referred to (Table IV.) as noticeable in *Asterias* and the two worms *Nereis* and *Chætopterus*. In one experiment in which a *Limulus* was kept for sixteen hours in fresh water the animal became so swollen that the gills burst and the water of the aquarium became blue from the hæmocyanin of the exuded blood. The blood of the animals subjected to diluted media became noticeably less viscous and owing to its increased volume and the high internal pressure, was easily obtained from the animal. When subjected to concentrated sea water it was often difficult to secure sufficient blood from the lobster to make the desired determinations. That an exchange of salts also takes place, although far less rapidly than the exchange of water, is shown by the fact that when the animals are placed in distilled water, chlorides are eliminated and a copious precipitate is obtained upon the addition of silver nitrate. No quantitative chemical analyses of the aquarium water were made but in one such case an increase in the osmotic pressure was indicated by the change in the freezing point; *Limulus* was the animal experimented with and after twelve hours' immersion the freezing point of the aquarium water had been lowered from — 0.02° to — 0.23°.

Quinton,[18, 19] experimenting with *Aplysia*, has also found an increase in weight when the animal is subjected to dilute sea water and a loss in weight in concentrated solutions, and he has further, by chemical analyses, found loss and gains in the amount of salts in the blood of this animal just equalling the respective gain or loss from the aquarium water. Frédéricq [3, 7, 8,] made similar analyses.

There are many other proofs of the permeability of the invertebrate membranes to various salts. Loeb's [15] experiments on the rhythmic contractions of medusæ (*Gonionemus*) indicade the permeability to NaCl, $CaCl_2$, and KCl. The death of invertebrates is easily induced by acids and the salts of the heavy metals. Frédéricq [9] has placed potassium ferrocyanide and nitrates in the aquarium water and later obtained positive tests for these substances in the blood of *Carcinus mœnus.* We may conclude then that the membranes of marine invertebrates as a class are permeable both to water and to salts and act exactly "like a dialyzer membrane."

The path taken by the exchanged material is not so certainly known. Frédéricq[6] assumes that the branchial membranes of *Carcinus mœnus* are the permeable structures but publishes no evidence supporting the view. Quinton[19] takes it for granted that it is the external wall which is permeable, and this seems to be true for *Aplysia*, the form with which he worked, as has been shown by Ph. Bottazzi and P. Enriquez.[2]

That the outer wall is the permeable structure of worms may be shown by a very simple experiment performed by the author on *Nereis* and *Chætopterus.* Ligatures were passed about the animals close to either end and drawn tight thus completely closing the alimentary canal; care was taken to avoid abrasion of the integument. When placed either in fresh water or dilute sea water swelling and increase in weight was obtained.

Limulus is an animal on which the permeability of the gills may be easily demonstrated. These structures are borne on the abdominal segment which may be bent ventrally to an angle of about 90°. When placed in this position and so propped up in the aquarium that only the abdomen is under the surface of the water, the mouth parts may be as much as fifteen centimeters above the surface. No water can enter the alimentary canal, nevertheless in equal parts of fresh water and sea water ($\Delta = 1.03°$), six hours sufficed to render the integument and gills tense and swollen. A freezing-point determination showed that Δ of blood had changed from $-1.82°$ to $-1.32°$. In another experiment with *Limulus* the animal was placed astride a narrow dish of fresh water and so supported that only the gills dipped beneath the surface with each rhythmic oscillation. After eight hours enough water had been absorbed to bring Δ down to $-1.41°$, the freezing point of the water had also changed from $-0.02°$ to $-0.20°$, and a copious precipitate of silver chloride was obtained. The gills of *Limulus* are permeable both to water and to salts. Metals in proteid combination, *e. g.*, copper of hæmocyanin, of course do not diffuse owing to the enormous size of the molecules with which they are incorporated.

Selachians (*Mustelus canis*). — Dog fish kept in fresh water for one hour showed signs of asphyxia and were removed in a dying condition. The defibrinated blood showed a considerable decrease in osmotic pressure, Δ being changed to $-1.45°$ C. After three hours' immersion in sea water diluted with an equal volume of distilled water the blood froze at $-1.60°$ C.

These experiments demonstrate the permeability of the membranes to water. As has already been pointed out, the composition of the selachian blood is very different from sea water in its salt content, but the osmotic pressure is maintained by the presence of large quantities of urea in the blood. It is evident then that selachian membranes are semi-permeable. Little more than this can be said, for this group has not been sufficiently investigated. The same may be said of the cyclostomes. Since these animals are found in both fresh and salt waters, and some species migrate at certain seasons from salt into fresh water, the author is making a more careful study of these groups. Mosso[17] found that selachian red-blood corpuscles were laked in a 2.5 per cent. solution of sodium chloride, and that when a selachian (*Scyllium*) was placed in fresh water death resulted in a few hours. He describes a disintegration of the blood corpuscles with the formation from their débris of a sort of coagulum which plugs up the branchial arteries with consequent death from suffocation. Death resulted in one half hour if the tails had been cut off before immersion in the fresh water.

Teleosts. — In nature we have experiments of the sort under consideration, in the movements of such fish as the eels and salmon, which go from salt into fresh water at the spawning season. We have as yet no data relative to changes in osmic pressure of the blood coincident with these migrations. The author experimented with *Augnilla chrysypa.* This form lives equally well in salt water or fresh-water aquaria and tolerate sudden transmission from one medium to the other without apparent injury. In testing the freezing point of the blood in different media variations were found, but it was impossible to attribute them to the actual changes in osmotic pressure, for similar variations were found in different tests made with animals taken from the same medium. In all these experiments it was necessary to sacrifice several animals to get even a minimum amount of blood for making determinations, so that the results were on the whole unsatisfactory; still, they suffice for the conclusion that only a slight, if any, change is induced by a change in the osmotic pressure of the external medium. The animals are "homoiosmotic."

Fundulus heteroclitus is a hardy little fish well suited to this form of experimentation, although the quantity of the blood is too small to admit of making cryoscopic determinations. It was found that if care was taken to select individuals which were not injured in catching, about eighty per cent. lived in fresh water for six weeks when the experiment was discontinued. This is as high a percentage as can be kept alive in the sea water aquaria of the laboratory. When placed in external media of concentrations varying from fresh water to sea water concentrated to twice its normal strength they showed the same hardihood. It is reasonably certain that the osmotic pressure of the blood does not change to any marked degree for an examination of the blood corpuscles did not show either laking in the dilute media or crenation in the media of higher concentration. The integument and gills are therefore impermeable. Loeb [14, 16] has found that *Fundulus* embryos will live in distilled water and in sea water to which 5 per cent. NaCl has been added.

The view that the membranes of these fish are completely permeable (Brown) [25] is not tenable, at least concerning adult *Fundulus*, as is shown by the following series of experiments which were repeated often enough to assure the verity of the results.

A large number of healthy specimens were selected and about one half the body surface denuded of scales by gentle scraping with the edge of a scalpel, or the skin was removed over an area of one square centimeter on each side; then they were divided into three lots and placed respectively into fresh water, sea water diluted with an equal volume of distilled water, and normal sea water. Of those kept in fresh water in every experiment from eighty to ninety per cent. died within twenty-four hours while all died in less than thirty-six hours. In normal sea water the fish suffered a similar fate although death did not intervene so soon.

But of those kept in sea water of one half its normal concentration only three per cent. were dead at a time when all those in the other two media had died, and seventy per cent. were kept alive for four weeks, when the wounds were all healed and the experiments discontinued. In these experiments therefore, no deleterious effects obtain when the internal and external media are approximately isotonic—in spite of the injuries and free interchange between blood and aquarium water. In the hypotonic and hypertonic solutions, however, distinct changes resulting in death, take place. In the strong solutions (normal sea water) microscopic examination showed that the blood corpuscles were crenated. In fresh water the fish became greatly swollen indicating the absorption of water. Whether laking or swelling of the corpuscles takes place was not determined in this series of experiments.*

From these experiments we may conclude that in all probability the blood of *Fundulus* does not suffer much if any change in concentration when the fish is transferred from salt water into fresh water or vice versa, provided the membranes are uninjured. If these experiments admit of general application to migratory toleosts they would indicate that these animals also are in some way protected from changes in the osmotic pressure of the blood and tissues and that the principal protective factor probably lies in a lack of permeability of their membranes. We may further conclude that in case of serious abrasion to the integument the membranes become permeable and a change of osmotic pressure of the blood results, a change which may induce the death of the animal. The great mortality of the salmon after spawning in the head waters of California's streams, is a well-known fact (Rutter[23]). Whether the generally battered condition of these fish at the spawning season bears any relation to changes in the osmotic pressure of the blood has not been investigated. It is not impossible that the actual cause of death lies in a decrease in the osmotic pressure of the blood and that the injuries are responsible for death only in so far as they permit the entrance of water and decrease the osmotic pressure of the blood. In consequence of such an event we would expect nutritional changes and possibly a disintegration of the red blood corpuscles. Such a disintegration in many marine teleosts has been described though the resistance of blood corpuscles of the migratory fish seems to be very much greater than of other forms of fish, for they lose their hæmoglobin only in salt solutions containing as low as 0.3–0.4 per cent. sodium chloride (Mosso[17]).

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*The hæmatocrit would doubtless prove a valuable aid in making experiments of this sort when it is impossible to obtain sufficient blood for freezing point determinations

The maintenance of an osmotic pressure lower than that of the sea water speaks for a relative impermeability of all the membranes of marine teleosts. The experiments we have described indicate an absolute impermeability of the integument. It may not be assumed that all the membranes are absolutely impermeable nor is there the same degree of impermeability in all teleosts as is indicated by the great variation in the resistance of marine teleosts to changes in the osmotic pressure of the aquarium water. Loeb's[14] experiments with *Fundulus* on the poisonous effects of sodium chloride indicate a certain degree of permeability of some surface. The poisonous action of heavy metallic salts, of acids, and alkalis indicate the same fact although many other factors must be taken into consideration among which is a possible and probable alteration of permeability due to these chemicals. A certain degree of permeability of young *Fundulus* is prettily shown by immersing them in sea water containing the merest trace of colored salts, such as salts of copper, cobalt and manganese. In these cases the solution may be almost colorless but in time the living embryos may become very deeply colored.

The facts indicate the probability of some regulative mechanism among teleosts (Höber[13]) the existence of which has not yet been demonstrated, and the nature of which cannot be affirmed.

Summary.

1. The sea water at Woods Hole, Mass., freezes at − 1.82° C., at Pacific Grove, Cal., Δ is − 1.90° C.

2. The blood or body fluid of a marine invertebrate has the same freezing point as the sea water from which it is taken, and therefore has the same osmotic pressure. This is also true of selachian blood, although the salt content is lower than that of

the sea water, the deficit of salts in the blood of this latter group is compensated by the osmotic pressure of the urea in the blood.

3. The osmotic pressure of teleost blood is about half that of the sea water ($\Delta = -0.8^\circ$ to -0.96° C.).

4. A dilution or concentration of the aquarium water always causes an equivalent change in the blood of invertebrates, and osmotic equilibrium between "internal and external media" is established. Their membranes are completely permeable. This permeability is proven for the integument of worms and the gills of *Limulus*.

5. Dilution or concentration of the aquarium water causes a change in the same sense in the blood of selachians, but death ensues before osmotic equilibrium is established. The membranes of selachians are semi-permeable.

6. Only slight if any change takes place when teleosts (*Anguilla*) are transferred from salt to fresh water and *vice versa*. Normal *Fundulus heteroclitus* will live in water varying in osmotic pressure from that of the tap to sea water concentrated to double its strength. The membranes of teleosts are impermeable, or the fish possess some regulative mechanism which keeps the osmotic pressure of the blood nearly constant. Extensive abrasion of the skin of *Fundulus* results in death in aquarium water of less or greater osmotic pressure than that of their blood, for example they die in fresh water and in *normal* sea water but not in sea water diluted with an equal volume of distilled water.

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BIOLOGICAL BULLETIN

FORM-REGULATION IN CERIANTHUS, IX.

Regulation, Form, and Proportion.

C. M. CHILD.

Regeneration and Proportion.

In the second paper of this series (Child, '03*b*) attention was called to the fact that the amount of regeneration is not proportional to the size of the piece. Small pieces above a certain limit regenerate as rapidly as pieces many times their size up to a late stage. Finally the small pieces fall slightly behind those of large sizes, probably as was suggested, because of the exhaustion of the material available for regeneration under the particular conditions (relative exhaustion, Child, '03*b*). Evidently the exhaustion will occur earlier, other conditions remaining the same, as the size of the piece decreases. Consequently there is some difference in the amount of regeneration according to the size of the piece, but this difference appears only at late stages and is very much less than the difference in size between the pieces. In other words the regenerated structures of small pieces are always relatively larger than those of large pieces.

The two series 54 and 55 which were described fully in my second paper (Child, '03*b*) illustrate this fact so well that it is desirable to recall certain points in this connection.

In the preparation of the two series the disc and œsophagus were removed by a transverse cut just aboral to the œsophagus and then the remaining portion of the body was cut into two pieces *A* and *B*. In Series 54*A* the oral piece was about four times as long as *B* at the time of section, while in series 55 the aboral piece *B* was about four times as long as *A*. Ten specimens were used for each series. In the pieces *A* of the two series we have pieces ending orally at the same level of the body, but of very different lengths. For purposes of direct comparison as regards rapidity and amount of regeneration pieces must always end at the same level of the parent body for the rapidity and amount of regeneration differ markedly with the difference in level (Child, '03*b*).

Comparing 54*A* and 55*A* it was found that, although the former pieces were about four times as long as the latter, the regeneration at the oral end was the same in both during more than a month. Finally in the latest stages the shorter pieces fell slightly behind the others, but even then the difference in length of tentacles in the two sets was only 1–2 mm. Figs. 1 and 2[1] represent diagrammatic sections of 54*A* and 55*A*, thirty-one days after section, when regeneration was about completed — the experiment was performed during December and January, consequently the total amount of regeneration was much less than in the summer specimens. The length of the tentacles in the figures is the average length for the pieces of each set, but since there was little difference in individual pieces the figures would serve equally well to represent almost any of the pieces of the two sets. The marginal tentacles in the pieces 54*A* (Fig. 1) were 7–8 mm. in length, while those of 55*A* (Fig. 2) were 6–8 mm. in length, *i. e.*, in some of the shorter pieces the tentacles were slightly shorter than in the long pieces, probably in consequence of the relative exhaustion of these pieces (see Child, '03*b*). The labial tentacles show scarcely any perceptible difference; the average length of these in 54*A* is 1.25 mm. and in 55*A* 1 mm. or a little more, but as these vary more

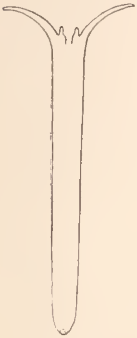

FIG. 1.

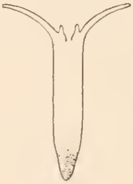

FIG. 2.

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[1] All the figures of this paper are diagrammatic outlines drawn from measurements of the living specimens. The stippled region at the aboral end represents the aboral new tissue. It is represented as if the body were seen in surface view, not in section, in order to indicate the shading in color between new and old parts and the absence of a sharp distinction. The oral end of the body is represented in section but the œsophagus is not drawn in most cases since it was usually impossible to determine its length in regenerating specimens.

in a single piece than do the marginal tentacles the very slight difference is not important.

Aborally the amount of regeneration was much greater in the smaller pieces 54*A* than in the larger 55*A* in consequence of the difference in position or level of these ends with respect to the animal from which they were taken (Child, '03*b*). Considering the relative amount of regeneration in the two pieces we find that it is much greater in the smaller pieces. But the important point for present purposes is the absence of proportionality.

Another series, Series 46, affords similar results. In this series disc and œsophageal region were removed from twelve large specimens by a transverse cut just aboral to the œsophagus. Four of the pieces (Ser. 46*A*) were kept without further operation, four others were cut in half and the distal half used (Ser. 46*B*) and from the oral ends of the remaining four small cylindrical pieces about 5 mm. in length were cut (Ser. 46*C*). Thus three sets of pieces were obtained all with oral ends representing approximately the same level of the original specimens. The pieces *A* were about four fifths of the total length of the body, the pieces *B* about two fifths, and the pieces *C* about one fifteenth or less. During the earlier stages of regeneration there was no appreciable difference in the rate or amount of regeneration in the three sets except as regards two pieces of *C* which broke up into smaller pieces and did not regenerate at all, a common phenomenon in pieces near the lower limit of size. Five weeks later when regeneration was complete the marginal tentacles of the pieces *A* were 7–8 mm. in length, those of *B* 6 mm. and those of *C* 4–5 mm. The differences in the labial tentacles were similar. While the length of the tentacles in the smaller pieces is not as great absolutely as in the larger it is relatively much greater. The pieces *C* were only about one twelfth the length of the pieces *A* but they regenerated tentacles more than half as long. Moreover, if we compare these pieces with the entire animals in which the marginal tentacles vary in length from 25–35 mm. we find that pieces one fifteenth of the size of an entire animal and taken from a region some distance from the oral end, *i. e.*, from a region where the power of regeneration is less than at the oral end, regenerate tentacles one seventh of the maximum length. However much the small pieces may elongate and decrease in transverse diameter the tentacles remain "too large."

Various other series of my experiments afford similar results, but these two cases are sufficient as illustrations.

Stated in general terms the conclusions drawn from the experiments are as follows: as the size of the piece decreases the relative amount of regeneration increases — provided of course that the level at which regeneration occurs is the same in all cases. It follows from this that the form, *i. e.*, the proportions, of regenerated specimens may differ widely from the typical form and that either reduction of the regenerated parts or extensive new growth in the longitudinal direction must occur. Reduction of the tentacles occurs, as has been noted (Child, '04*c*) but it is due to change in external rather than internal conditions. If the pieces are fed they will of course in time attain approximately the typical proportions. But the important point is that the regenerative processes in absence of food are not proportional.

I am inclined to believe that these facts indicate that the stimulus to regeneration is as great in small as in large pieces; that the difference in absolute amount of regeneration is due to the earlier relative exhaustion of the smaller pieces, *i. e.*, the absence of energy available in the presence of a given stimulus; and that finally if this material could be supplied in such manner as to be available only for the regeneration of a particular set of structures, *e. g.*, the tentacles, they would continue to grow until equilibrium resulted between formative stimuli and destructive factors, no matter what the size of the piece, provided it was above the minimum. In other words, it is probable that the decrease in the absolute amount of regeneration in *Cerianthus* with decrease in size of the piece is due merely to decrease in amount of available energy and not to any inherent capacity for proportional regeneration. Furthermore, it is probable that various other cases of so-called proportional regeneration are in reality similar in nature to this case.

Regulative Changes of Proportion in the Old Parts.

The next subject which requires consideration is the change of form of pieces as a whole. A cylindrical piece cut from the body is of course relatively shorter and less slender than the typical animal. From this piece tentacles regenerate at one end and more or less growth of new tissue occurs at the other, but neither of these is proportional to the size of the piece. It has already been shown that the oral regeneration is too great in amount to permit the reduction of the piece to the typical proportions, but there remains the further question as to whether the piece shows any approximation to typical proportion, or in other words whether changes in proportion of the old parts of the piece occur which can be interpreted as constituting an approach to the typical form.

In order to answer this question careful measurements of the pieces repeated at frequent intervals are necessary, but measurement is a difficult and uncertain matter in connection with *Cerianthus.* The form varies so greatly with different degrees of distension and with the different activities of the animal that it is impossible to obtain anything more than a rough approximation. I have, however, carried out several series of measurements extending over two months or more and have succeeded, I think, in minimizing some of the sources of error. In making these measurements various precautions were observed; the pieces were absolutely undisturbed for several hours, or in most cases for nearly a day before the measurements were made, since it is only in this way that anything like complete distension and elongation can be obtained. Measurement was made by immersing a small millimeter measure in the water with the specimen, care being taken not to touch it, for a touch is likely to cause instant contraction. All measurements were repeated several times at intervals during the day, for there is no certainty that the animal is fully distended at the time of a given measurement. From these different measurements the maximum series was selected as showing the largest size attained by the specimen during a given day. The following dimensions were measured for each piece so far as the parts designated were present; the whole length of the piece from disc to aboral pore; the length of the marginal tentacles; the length of the labial tentacles; the length of the aboral outgrowth of new tissue; if such were present; the diameter of the disc, *i. e.*, the distance between bases of tentacles on opposite sides; the diameter about half way between the disc and the middle of the piece, designated as oral diameter;

the diameter at the middle in some cases where it differed from the oral diameter; and finally the diameter about half way between the aboral end and the middle, designated as aboral diameter. At the same time diagrams were drawn showing any pecularities or special features of the regeneration which might occur. All the figures of this section were drawn from these measurements and diagrams. In cases where the tentacles differed markedly in length on different parts of the circumference the two tentacles of the figure represent the extremes.

No one can be more fully aware than myself that these measurements are not and cannot be more than rough approximations. Direct measurement of the labial tentacles was often impossible, but an estimate of their length was made in such cases by placing the measure as near to them as possible and making careful comparison. It is probable, on the other hand, that these measurements are as exact as the instability of form in *Cerianthus* will permit us to obtain. While I do not believe that extended investigation along this line is profitable in this case in consequence of the absence of stable form I have proceeded far enough to convince myself that such measurements are unable in a general way. Marked differences can certainly be detected.

The results obtained from the several series of measurements made agree in general though they showed some differences in detail, and I think it is possible to draw certain conclusions from them. The following series is selected as an example from among those made.

Series 36.

On October 20, 1902, disc and tentacles were removed from a large specimen by a transverse cut, and the body was then cut into four pieces, *A*, *B*, *C*, *D* (Fig. 3). These pieces were measured at intervals of two or three days during about six weeks.

The following table is an abstract of the measurements. The proportions of the whole animal before section, the estimated lengths of the pieces cut and the dimensions of these pieces on five different dates during the six weeks are given in millimeters. These are sufficient to show the general trend of the changes and measurements made in the intervals between these dates have therefore been omitted as unnecessary for the present purpose.

Date.	Piece.	Length of Body.	Length of Marginal Tentacles.	Length of Labial Tentacles.	Length of Aboral New Tissue.	Diameter of Disc.	Oral Diameter of Body.	Middle Diameter of Body.	Aboral Diameter of Body.	Figure.
Oct. 20, '02. Before Section.	Whole.	95	30	12–15		12	7	6	5	Fig. 3
Oct. 20, '02. At time of Section.	*A*	25								Fig. 3
	B	28								" 3
	C	12								" 3
	D	25								" 3
Oct. 21, '02.	*A*	9					5		6	Fig. 4
	B	15					6		6	" 9
	C	5					5		5	" 14
	D	10					5		3	" 19
Oct. 29, '02.	*A*	15	1–1.5				6	7	6	Fig. 5
	B	20					5	6	6	" 10
	C	6					4		4	" 15
	D	22					4	6	4	" 20
Nov. 6, '02.	*A*	10–12	7–8	0.5–1	1	6.5–7	6		6	Fig. 6
	B	18	4–5	0.5		6	5		5	" 11
	C	8–9	0.5–2			5	5		4.5	" 16
	D	18	0.5			4.5	4.5		4	" 21
Nov. 20, '02.	*A*	18	8–10	2–3	3	6	5		3.5	Fig. 7
	B	27	10–12	3–4	3	6.5	4		4	" 12
	C	10	5–8	1	2	5	4		4	" 17
	D	25	8–10	2		5	4		3	" 22
Dec. 2, '02.	*A*	27	12	3–4	3–4	6–7	4.5		3.5	Fig. 8
	B	28–30	12–14	3	3	6	4.5		4	" 13
	C	18	8–9	2–3	2	5	3		2.5	" 18
	D	25	11–12	3		5	3.5		3	" 23

A horizontal reading of the table gives the measurements for each piece at each date and the last vertical column headed "figure" gives the number of the text figure representing the piece at the stage indicated by the horizontal division.

It is readily seen from the table and figures that the length of each piece gradually increases while its transverse diameter gradually decreases during the course of the experiment. The length of the tentacles increases during the whole period. There are several irregularities in the table which are undoubtedly due to the great changes in form in individual specimens: for example, piece *A* is shorter on November 6 than on October 29. This difference is merely temporary and due to the fact that the piece is not as fully extended at the second measurement as at the first. But the general result is sufficiently clear from both table and figures. The extremely small size of the specimens on the day after section is of course due to complete collapse. The pieces in this condition are not strictly comparable to the distended pieces.

The data indicate that a marked change in form occurs in these pieces and that this change consists in an approach to the typical proportions, although none of the pieces attain them. For example the piece *B* (Fig. 13) approaches most closely at the end of the experiment to the original proportions of the whole before section, but even here the tentacles are relatively longer, the body-length is relatively less or the transverse diameter is relatively greater than in the whole. In the short piece *C* (Fig. 18) the proportion between length and transverse diameter is 6:1

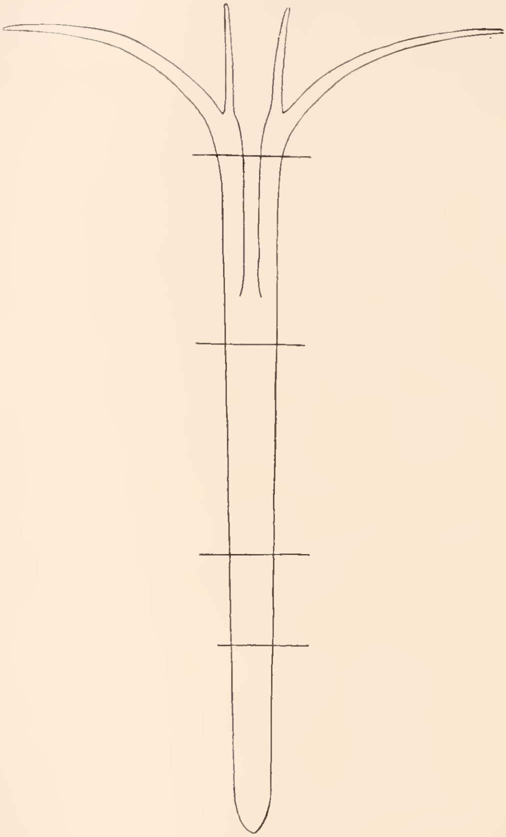
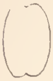


Fig. 3.

as compared with about 13.5:1 in the whole. The relation between length of marginal tentacles and length of body is about 1:2 while in the whole it is 1:3. The change in the relation between length and transverse diameter in each piece during the experiment consists chiefly in decrease in the latter. None of the pieces except *C* have increased in length to an amount equal to the growth of new tissue at the aboral end. In other words all but *C* both length and transverse diameter of the old tissue have decreased but the latter much more rapidly than the former. In the actual decrease in size two factors are probably concerned, viz., loss of substance and decrease in general internal pressure: of these the former is due to starvation and use of material in regeneration, the latter both to approaching exhaustion and to the reduction in temperature which occurred during the course of the experiment from October to December; both of these conditions reduce ciliary activity and therefore internal pressure also. But these factors alone must bring about a proportionate or nearly proportionate reduction in form. Other factors must therefore be concerned in the change of form which occurs in these pieces.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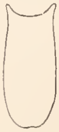
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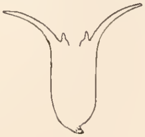
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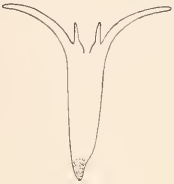
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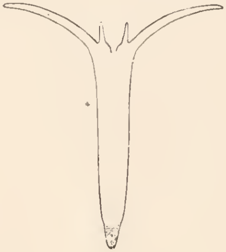
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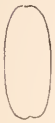
It remains then to determine whether or not this change of form is due to some inherent capacity of the protoplasm independent of external conditions. The first point of importance in the consideration of this question is the dependence of the change of form upon distention with water. Pieces kept open at one or both ends continue indefinitely to grow shorter and smaller until finally they form rounded masses, even though originally the length was many times the diameter; in short the changes in these collapsed pieces are opposite in character to those that occur in the pieces described above. The reduction of collapsed pieces has been mentioned several times in this series of papers (see especially '04*b*) and need not be discussed in detail again. The fact that the relation between length and transverse diameter changes in opposite directions in collapsed and distended pieces is important and may, I think, be taken to indicate that the distention with water plays some part, either directly or indirectly, in the "elongation" which distended pieces undergo. Two possibilities suggest themselves to me in this connection, viz.: either the pressure of the water in the enteron constitutes in some way a stimulus to elongation or the change of form is an incidental result of the behavior which is markedly different in distended and collapsed pieces.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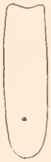
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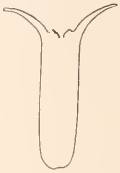
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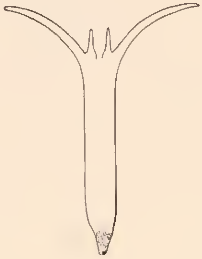
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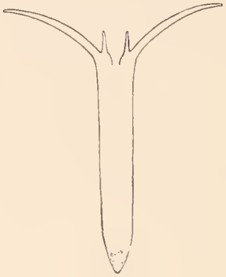
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Regarding the first of these possibilities, viz., internal pressure as a stimulus to elongation I can offer no experimental evidence. I desire merely to call attention to certain points. It is evident that the general internal pressure cannot constitute a stimulus to elongation unless we postulate a difference in the reactive capacity of the tissues in different directions; it may be that such a difference exists but I know of no basis of fact for this assumption. On the other hand it is possible that currents in the enteron might exert local pressure in particular directions. It has already been noted in numerous cases (Child, '04*b*, '04*c*, '04*d*, '04*e*, '05) that there is some ground for the belief that circulatory currents (Child, '04*b*) may constitute factors in the localization of regeneration, *i. e.*, that the local pressure may act as a stimulus to growth. Currents passing orally along the inner surface of the body wall in all the intermesenterial chambers must produce a total pressure of considerable amount upon the oral end. On the other hand a current passing aborally along the inner margins of the mesenteries or a part of them must finally strike the aboral end and produce pressure there. Now the effect of these currents may conceivably show itself partly in local growth in region of impact, *i. e.*, where the tension upon the tissues resulting from the impact of the current is greatest and partly in a diffuse growth or a change of form in accordance with the tension in the surrounding regions to which some degree of the tension is transmitted. Since these currents strike the terminal regions of the body local growth at the ends and elongation of the whole must be the result if they are effective in the manner suggested. In pieces kept without food the elongation must be accompained by a reduction in the transverse diameter. These suggestions as to the effects of circulatory currents, like those made in previous papers (Child, '04*b*, *et seq.*) must be regarded at present as merely tentative. They may be entirely incorrect but I think the hypothesis of the effectiveness of circulatory currents as localizing formative stimuli cannot be rejected off-hand. It serves so well as a basis for explanation of various phenomena that it must at least receive consideration. I hope at some future time to obtain experimental evidence regarding this hypothesis.

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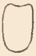
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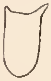
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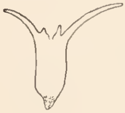
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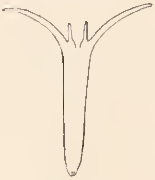
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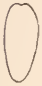
The second possibility, viz., that the change of form may be an incidental result of the movements of the animal must also be considered. As noted above, the behavior of typically distended specimens is very different from that of the same pieces during collapse. The creeping habit of *Cerianthus* is well known ; the animals are able to move about freely, lying upon the side and moving with either oral or aboral end in advance. They move not only on horizontal surfaces, but are able to climb the vertical sides of a glass jar or aquarium. In my aquaria specimens often climbed two feet or more up the vertical side. Cilia afford of course the motive power and the slime secreted serves as a means of adhesion to surfaces. Observation of creeping pieces shows clearly that the body is subjected to more or less tension in the longitudinal direction during this movement. Probably the slime secreted by the ectoderm is responsible for most of this tension. I think it possible that this tension may bring about some degree of elongation in much the same way as in *Stenostoma* (Child, '02, '03).

In *Cerianthus* the tension is due rather to the slime secreted than to the use of the end as an organ of attachment, as is the case in *Stenostoma*, but the result, viz., longitudinal tension upon the tissues, is similar in both cases. If the tissues here are affected by the tension as they are in the turbellaria, elongation must result, whether from growth or from mechanical rearrangement of the tissues or cell-elements.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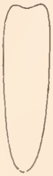
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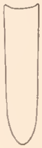
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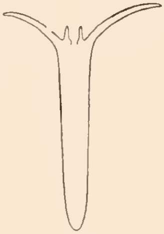
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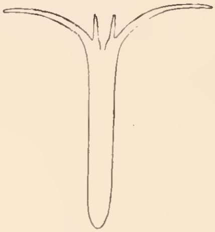
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Soon after beginning my experiments I found that collapsed specimens and pieces in early stages of regeneration crept about very little. It was found that such pieces could be kept without danger of loss in Stender dishes with sides 7–8 cm. in height, which were placed on the bottom of large tanks filled with water; the pieces never crept out. Later, however, the same pieces would creep out of battery jars with sides 30–40 cm. or more in height. In these respects I noted a difference between those pieces which still retained the original oral end and tentacles and those in which oral structures were in process of regeneration. Pieces of the first sort began to creep about soon after closure of the aboral end and distension occurred, *i. e.*, within a few days in many cases, while the pieces with regenerating oral ends usually moved very little during the first three to four weeks after section, *i. e.*, before regeneration of the oral structures was well advanced. In short, the collapsed specimen and the specimen — collapsed or not — in which the oral structures are not well developed, do not exhibit the typical behavior of the species as regards movement. Probably in the one case the absence of distension brings about some change in the internal stimuli which inhibits the motor impulses to a greater or less extent. In the other case the most highly organized portion of the body — the oral region — is absent and the reactions of the pieces are much less vigorous and effective.

To sum up: two possible factors in the elongation of pieces have been suggested; the one, tension upon the terminal regions of the body in consequence of the impact of enteric circulatory currents against the wall; the other, tension upon the tissues in the longitudinal direction in consequence of movement over surfaces accompanied by some degree of adhesion.

It is impossible to determine at present whether one or the other of these factors is chiefly concerned, or whether both play important parts in the change of form. It may be that other unknown factors are concerned, but it is highly improbable that there is any mysterious internal ultra-physico-chemical factor which brings about a characteristic form. Mention has already been made of the fact that collapsed pieces never undergo this change of form, but depart farther and farther from the "typical" form. Now it is possible to keep a specimen collapsed by means of a very small opening at one end or elsewhere, provided this be reopened frequently. It is difficult to understand how such an opening should interfere with any internal or "vitalistic" factor capable of causing return to the typical form. Here again experiment indicates the impossibility of explaining the facts with the aid of Driesch's hypothesis (Driesch, '01, '03).

Both of the hypotheses suggested belong in one category, viz., that of mechanical tension. Whether this tension brings about simply mechanical deformation of the tissue elements, or whether it acts as a functional stimulus to which the tissues react by growth, cannot readily be determined. Probably both mechanical deformation and growth occur; indeed, it is probable that the former brings about the latter.

According to this view the elongation of pieces of *Cerianthus* and the extremely elengated form of the body of normal animals are due to the same factors.

The tubicolous habit of the animal may perhaps be regarded by some as responsible in some degree for its elongated form. In the tube only longitudinal increase in size can occur. The animal frequently leaves its tube, however, and burrows in another spot; the size of the new tube will be determined by the size of the animal in each case. Moreover, I do not think the reduction in transverse diameter observed in pieces can be explained in this manner. The slime which encloses the bodies of specimens kept in clear water is not sufficiently elastic to reduce the diameter. In so far as the burrowing tubicolous habit is correlated with the creeping habit, it may constitute a factor in the production of the characteristic elongated form.

One point still requires brief consideration. The table and the figures indicate that piece *C* undergoes a much greater change of form than the other pieces. It is the only piece in which the length of the old part is greater at the end of the experiment than at the beginning, *i. e.*, provided the measurements are correct. There is of course uncertainty regarding all the measurements but if we suppose this difference between *C* and the other pieces to be real it requires explanation.

In my study of *Stenostoma* (Child, '02, '03) it was shown that the change in direction of the tension upon the tissues is an important factor in change of form. In a short piece of *Stenostoma* using its posterior end as an organ of attachment the directions of the various strains to which the tissues are subjected differs much more from those to which they were subjected as a part of the whole than would be the case in a long piece. This must be true in all cases except where the posterior end of the piece formed the posterior end of the whole. In that case the directions of the strains remain as before. For further consideration of the case the reader is referred to the above-mentioned paper. The point of importance for our present purpose is that the short piece must undergo greater change of form than the large piece except when it is taken from the posterior end.

We can I think, apply the same course of reasoning to *Ceri-*

anthus. If we consider animals moving about, oral end in advance as they very commonly do, a cylindrical portion of the body situated at any region except the extreme end, is, let us assume, subjected to a certain degree of tension in consequence of the movement. Now since this piece is attached to other parts both at its oral and aboral ends about its whole circumference the strains to which its tissues are subjected are almost wholly parallel to the longitudinal axis. If now the piece be removed from the whole and allowed to regenerate in the typical manner closure of the ends and outgrowth of new tissue at the aboral end occur before the piece begins to move about. If now the piece begins to creep and is in consequence subjected to tension the elements of this tension affecting various parts will differ not only in amount but also in direction because the piece is no longer attached to other parts at both ends by its whole circumference. Comparing a long and a short piece it is clear that the change in direction of the strains in the long piece considered as a whole is very much less than in the short piece. We may expect, therefore a greater change of form in the short piece than in the long piece, after equilibrium is attained. The greater change in the piece *C* as compared with *A*, *B*, and *D*, which I am inclined from comparison with other pieces to regard as real, may then be explained on the same basis as similar changes in other creeping forms. The fact, shown by the figures and table, viz., that the change of form became much more rapid toward the end of the experiment, *i. e.*, in the fifth and sixth weeks, agrees well with the fact that the piece did not begin to move about freely during the first month after section. During this time it never climbed out of the jar, but during the next two weeks it climbed out of a deep jar into the large tank almost daily and wandered about the tank. As often as found outside it was returned to the jar. It was during this time that the chief change of form occurred (compare Figs. 17 and 18). These facts may perhaps indicate that the movements are more important than the internal pressure in bringing about the change of form.

In *Cerianthus* then we find another case in which the assumption of a mysterious formative principle is in no way necessary. Form in the lower animals is probably to be considered as relatively simple. It should be borne in mind, however, that cells and tissues of a certain constitution, *i. e.*, *Cerianthus*-protoplasm, must be acted upon in order that a certain result characteristic of the species may be obtained. In another species the result of a stimulus similar in degree and kind may be more or less widely different. At best the problem of form is complex but I believe that in general we must look to function for an explanation of form, whether direct or indirect, and not to form for an explanation of function. This opinion does not involve the paradox of differentiation of function in a structurally isotropic protoplasm, but requires a distinction between chemical and physical structure of protoplasm and morphological structure in general. Function in a general sense is an expression and result of the first, while the second is, at least in many cases, an expression and result of function.

Experimental Duplication of Parts.

By partial longitudinal splitting of the body, the separated portions remaining attached to the undivided part, it was possible to produce specimens with two oral or aboral ends as the case might be. In cases where one of the parts separated by the longitudinal cut was considerably smaller than the other, and especially in cases where the cut was somewhat oblique, the smaller part or that attached to the undivided region by the narrower portion very commonly separated from the remainder of the body within a few days and regenerated independently. In many cases also closure was much delayed by the irregular inrolling of the edges and one or both of the split portions became greatly reduced or constricted off. In a certain number of the pieces, however, the split portions roll longitudinally and their edges unite thus giving rise to the duplication. In case of duplication of the oral end the two parts are in reality "halves," each possessing approximately half the full number of tentacles. Where closure is perfect or nearly so each disc of course possesses a mouth and œsophagus formed by union of the longitudinal cut surfaces of the half œsophagus — in reality half structure. Whether in cases where one of these half structures contains the old siphonoglyphe the other ever forms a new one I do not know. I have never found a new one, but it may appear in time.

In cases of aboral duplication each part is really a "half," but the aboral pore is duplicated, each pore being formed from a part of the old pore in case the cut passed through it, or one pore being formed anew in case the cut passed more or less to one side of the old pore.

The varieties in method of closure in these cases are almost infinite as may be conceived from the discussion of inrolling in a previous paper (Child, '04*a*). Scarcely any two of the resulting forms are alike but the many differences in detail do not add essentially to our knowledge, being merely illustrations of one general principle. They do show very clearly, however, how little power the animal possesses to maintain or return to a particular form after section. All is pure chance in the matter of closure. Whatever portions of the cut surfaces come into contact unite. The tensions due to internal water-pressure may serve in some cases to modify the peculiar forms, but in other cases this does not occur. All depends upon the conditions of the particular case.

In cases where closure and duplication of the oral or aboral ends is accomplished I have never seen reduction, absorption, or loss by constriction of one of the two parts afterward. Occasionally if the duplication involves merely the extreme end it gradually disappears; the two halves gradually becoming a whole. In other cases where the duplication extends for some distance from the end so that the two parts are distinct, I have not noted any changes which might be interpreted as a regulation of the atypical form, although specimens of this kind have been kept for more than three months.

There is, no doubt, that many other varieties of monstrous forms may be produced. The only obstacle is the frequent separation of parts from the body after severe operation.

SUMMARY.

1. Regeneration in *Cerianthus* is not proportional to the size of the piece. The smaller the piece, other conditions remaining the same, the greater the relative amount of regeneration. As regards absolute amount of regeneration the small and large pieces are at first alike, but later the small piece falls behind, *i. e.*, regeneration is retarded and ceases sooner than in the large piece, probably owing to lack of available energy.

2. Cylindrical pieces usually undergo a greater or less change of form during or after regeneration: this consists of an increase in length and a decrease in transverse diameter.

3. The change is slight during the earlier stages of regeneration before the oral structures are developed. It seems probable that it is the result, either directly mechanical or reactive or both of longitudinal tension upon the tissues. The tension in turn may be due in part to internal circulatory currents and in part to the habit of creeping over surfaces in the direction of the longitudinal axis.

4. In pieces maintained in the collapsed condition the change of form is the reverse, *i. e.*, the length decreases and the transverse diameter increases, at least relatively.

5. It is possible to produce forms with duplicated oral or aboral ends by partial longitudinal splitting: In cases of oral duplication each disc is essentially a "half" structure or fractional structure, bearing approximately the number of tentacles corresponding to the portion of the circumference which it represents. At the aboral end a new aboral pore may be formed in case the cut does not pass through the old pore.

6. No marked regulation, reduction or absorption of these duplicated structures has been observed except occasionally in cases where the duplication included only the extreme terminal portions.

7. The results of attempts to produce duplications and abnormal forms depend largely upon chance. Whatever portions of the cut surfaces come into contact unite and many peculiar forms result which may be more or less modified in some cases by the tensions and pressures to which the tissues are subjected.

HULL ZOÖLOGICAL LABORATORY, UNIVERSITY OF CHICAGO,
December, 1903.

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NOTES ON THE LIFE HISTORY OF THE STYLOPIDÆ.

CHARLES THOMAS BRUES.

During the past summer I had the opportunity of making a number of incidental observations on the two Texan species of *Xenos*, and of discovering the female of one of them, which has not hitherto been described. As these notes throw some light on the life history of these interesting creatures, I venture to present them at this time.

On May 22, at Paris, Tex., I captured a large overwintered female of *Polistes rubiginosus* which had evidently just left its hibernation quarters. Examination showed that it harbored a single female specimen of *Xenos nigrescens* Brues. The head of the parasite protruded between two of the apical abdominal segments and a number of triungulins were emerging from the small rectangular orifice on the anterior portion of the exposed surface of the head. The triungulins do not move very rapidly and cling rather tenaciously to the body of the wasp, even when the latter is shaken violently about. They keep continually in motion, however, and when the wasp is resting a number of them are always crawling off upon adjacent objects. They are rather dark brown in color and scarcely visible to the naked eye. The following description is drawn up from a number of mounted specimens:

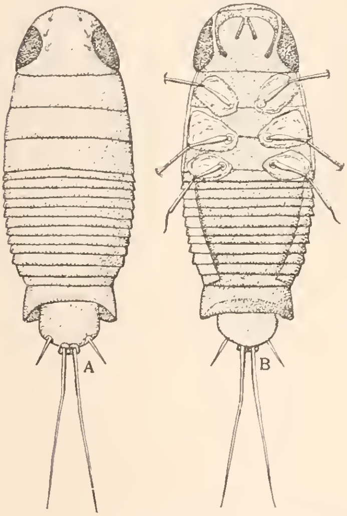

FIG. 1. Triungulins of *Xenos nigrescens* Brues. *a*, dorsal view; *b*, ventral view.

Length .28 mm. Oval, head obtusely rounded anteriorly. Head and thorax together slightly longer than the abdomen. Head a little less than half as long as the thorax, almost semicircular when seen from above, being truncate behind. Eyes large, strongly pigmented. Oral opening large, almost contiguous with the eyes below; mouth parts consisting apparently of a short proboscis-like organ with chitinous sides. No antennæ or other tactile organs to be seen. Thorax one and one-half times as long as wide, consisting of three nearly equal transverse segments. Each segment below bears a pair of very small and delicate legs. The coxæ are all greatly swollen and globose, those of each side contiguous with one another and the pairs only moderately separated along the median line. Each coxa is hollowed out below and the trochanter sunk within it. Femora slender, enlarged somewhat at the base, bearing a spine apically. Tibiæ slender, of equal width, the hind ones with a preapical spine. Tarsi greatly reduced, those of the four anterior legs scarcely distinguishable from the tips of the tibiæ, furnished with a pulvilliform appendage; the posterior pair elongated, with a styliform appendage. Abdomen consisting of nine short, transverse segments and an elongated tenth segment which encloses the extrusible tip of the abdomen (11th segment?). The dorsal sclerites reach far down on the sides, as do also those of the thorax, making the underside of the body somewhat concave. Tip bearing two approximated bristles, each as long as the abdomen, in addition to a much shorter one at each lateral angle of the last segment.

The triungulins are strongly campodeiform in type, but show several remarkable adaptations in the shape of the legs. Aside from these peculiarities they are very similar to Meloid triungulins.

There are several differences to be noted between them and the triungulins of *Stylops* as figured by Packard ('98, 695) and Sharp ('01, 300). Principal of these is the presence of a pair of large, strongly pigmented eyes, and the structure of the legs in which the tarsi are so much reduced. In Packard's figure the different form of the anterior and posterior tarsi are shown somewhat as I have observed them in *Xenos*.

Of the three species of *Polistes* which are especially abundant in Texas, only two seem to be at all generally attacked by *Xenos*. These are *Polistes annularis* and *P. rubiginosus*. From the former both *Xenos pallidus* and *X. nigrescens* were bred in considerable numbers, and from the latter only *X. nigrescens*, and that in smaller numbers. The fewer *Xenos* obtained from the specimens of *rubiginosus* is due both to the smaller percentage of individuals infested and usually to the occurrence of only one parasite in each wasp. In *annularis* there were on an average from three to four *Xenos* in each parasitized wasp, and in one case ten fully developed male pupæ. Although hundreds of specimens of *Polistes texanus* were examined they showed no parasites at any time during the season.

The following tabular arrangement has been prepared to show some of the more important relations betwen the *Polistes* and the parasites in two nests of *annularis*.

First nest of *Polistes annularis*; 86 female wasps, 44 of them parasitized by *Xenos nigrescens*. An examination of 36 of these revealed parasites as follows:

Wasp No.	Male *Xenos.*	Female *Xenos.*	Wasp No.	Male *Xenos.*	Female *Xenos.*	Wasp No.	Male *Xenos.*	Female *Xenos.*	Wasp No.	Male *Xenos.*	Female *Xenos.*
1	1	1	10	3	0	19	3	0	28	1	0
2	0	1	11	1	0	20	2	0	29	1	0
3	2	1	12	3	0	21	7	0	30	4	0
4	1	0	13	6	0	22	6	0	31	4	0
5	1	0	14	3	0	23	2	0	32	1	0
6	1	0	15	5	0	24	1	0	33	4	0
7	1	0	16	4	0	25	3	0	34	2	0
8	4	0	17	2	0	26	1	0	35	1	0
9	4	0	18	1	0	27	3	0	36	3	0

In the total of 36 wasps examined there were 91 male and only 3 female parasites. In two cases the females were associated with males in the same wasp. This great preponderance of males in the nest is very remarkable and may possibly be due to the season of the year (July) since the next nest to be described contained many more females and was collected much later (October 3).

Second nest of *Polistes annularis*; 42 female wasps, 36 of them parasitized by *Xeno pallidus*.

Wasp No.	Male *Xenos.*	Female *Xenos.*	Wasp No.	Male *Xenos.*	Female *Xenos.*	Wasp No.	Male *Xenos.*	Female *Xenos.*	Wasp No.	Male *Xenos.*	Female *Xenos.*
1	6^{e}[1]	1	10	0	2	19	0	1	28	0	2
2	6^{2e}[1]	1	11	0	2	20		2 larvæ	29	4	0
3	5	1	12	1^{e}[1]	0	21	0	1	30	4^{e}[1]	1
4	3	3	13	1	2 larvæ	22	1	2	31	0	1
5	10^{e}[1]	0	14	0	1	23	2	3	32	1^{e}[1]	2
6	4	2	15	2	1	24	0	2	33	6	2
7	1	2	16	1	2	25	2	1	34	0	1
8	2	0	17	1	3 larvæ	26	1	4	35	0	2
9	7	0	18	1	1	27	5^{e}[1]	0	36	4	1

Total males, 81. Total females, 44. Wasps with females only, 10. Wasps with males only, 8. Wasps with both males and females, 17. Wasps with larvæ only, 1.

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[1] *e* refers to the number of males which had emerged when the wasp was captured.

A study of the conditions in this nest bring to light a number of interesting points. It is seen that seventeen, or almost fifty per cent. of the wasps contained both male and female parasites, while about an equal number contained parasites of only one sex. In these latter the number infested by males and females was about equal. A single wasp contained only larvæ, while several bore larvæ in addition to imagines. The presence of larvæ in full-grown wasps is no doubt due to a failure to keep pace in development with the growth of the wasps.

From the large number bearing parasites of both sexes, in seems probable that at times the sex of the parasites is in no way influenced by the host, or rather, that the sex of the specimens to mature in a single wasp from the large number of *Xenos* larvæ usually present is not always the same as I had previously supposed from former observations ('03, 246).

A second point of interest is the considerable number of wasps, seven in all, from which males had emerged before capture. From previous observations upon wasps in captivity ('03) I was led to believe that the hosts died very soon after the emergence of male *Xenos* from their bodies.[1] Such did not appear to be the case here, for one wasp which was actively feeding upon the nectary of a cotton square late in the afternoon (the males almost always leave the pupa case early in the morning), proved on later examination to bear ten empty male pupa cases between the segments of the abdomen. In some of the wasps included in the table it is quite possible that a number of the males may have emerged at time of capture or during the several hours confinement of the wasps in a screen box before careful examination. It has been remarked by Packard that the males are apt to emerge during any excitement or great muscular exertion of the host, for example when the wasp is caught in a net. This may to some extent account for the discrepancy between the two series of observations.

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[1] In this connection it is interesting to note that this fact was commented upon in 1793 by Rossi in what is no doubt the first mention of *Xenos* in scientific literature. His statement given in the *Bulletin de la Société Philomathique*, Vol. 1, p. 49, is as follows: "Cet insecte habite à l'état de larve et de chrysalide dans la guêpe francaise *vespa gallica* C'est sous la quatrième anneau de l'abdomen de cette guêpe que se trouve sa chrysalide; sa présence ne nuit pas à la vie de la guêpe, et on rencontre souvent sous les anneaux de leur abdomen les chrysalides dont l'insecte est sorti, sans que les guêpes paroissent incommodées."

The presence of numbers of females in the wasps during October when *Polistes* are searching for hibernating quarters suggests that the parasites pass the winter in this state. With these species as is known to be the case with *Xenos peckii*, a cursory examination under the microscope showed that the masses of eggs in several females were in a very early stage of development. From this there seems no reason to doubt that the gravid females hibernate in this state, and that the later embryonic development is passed during the early part of the following spring. It would appear very doubtful that any males hibernate. The fact that many had emerged and that those still remaining in the pupal envelopes had already acquired their adult color by October first shows almost conclusively that all would emerge before winter. Spring observations also support this idea for no males were seen in over-wintered wasps. Neither were any wasps containing empty male pupal cases seen in the spring. During the winter a large proportion of the *Polistes* die, and no doubt the ones from which males had emerged would succumb first. In the nest in question, then, only ten of the parasitized wasps could be expected to survive the winter. That the majority of these die also is shown by the scarcity of parasitized wasps in the spring.

From these facts it is evident that no males survive the winter and probable also that no wasps which have contained males survive. This necessitates the death of a large proportion of the female *Xenos* since it has been noted (*ante*, p. 292) that of 44 females only 14 were in wasps that did not contain males.

On account of the difference of opinion as to the way in which the wasps treat the emerging male parasites, a close watch was made on several occasions, but at no time could it be observed that the wasps took any notice at all of the fluttering male *Xenos*.

Quite recently Pierce ('04) has added to our list a new species of Stylopidæ described by him as *Xenos pulvinipes*. An examination of his figures and description convince me that this form is generically distinct from *Xenos* for several important reasons. According to the description the female possesses distinct eyes, a character at once distinguishing it from *Xenos* and so far as I am aware from all other Stylopid females. It has also segmental spiracles which are absent in the other forms, and the males have very peculiarly modified tarsi.

Following is the description of the female of *Xenos pallidus* :

Xenos pallidus Brues ('03).

Female. Length 8–12 mm. Distinctly more slender than *X. peckii* or *X. nigrescens*. Head black, except on the anterior third, the lines between the colors straight, transverse. Head in outline more elongate than in the other two species and less bowed on the sides, widest quite near the posterior angles (see figure 2). Thoracic integument very dark, wrinkled as usual, its posterior margin gradually slanting backwards on each side toward the median line. Dorsal stripe very distinct, always extending over seven segments as shown by its constrictions, distinctly marked posteriorly and not fading out indefinitely. Openings to the oviducts distinct, four in number, one on each of the four anterior abdominal segments near the posterior margin. Abdomen more or less distinctly constricted just before its tip.

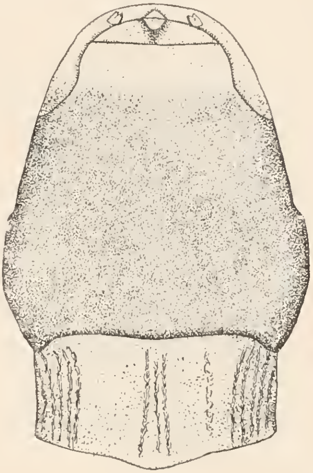

FIG. 2. Exposed portion of adult female of *Xenos pallidus* Brues.

Described from over 90 specimens found associated with the males of the species in the bodies of *Polistes annularis*.

In conclusion, I wish to thank Mr. F. C. Bishopp who collected a considerable portion of the stylopized *Polistes* considered in the foregoing notes.

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IMMATURE SPECIMENS OF PENELLA FILOSA.

M. T. THOMPSON.

The young stages of all parasitic Crustacea are of interest, and this is especially true with respect to the highly modified forms. Hence I was pleased to find among a number of *Penella filosa* loaned me by Dr. J. S. Kingsley, a few immature specimens. *Penella filosa* is the commonest species of *Penella* on our coast and in all probability is unhesitatingly to be identified with the form described from the sword-fish by Linnaeus in 1758 as *Pennatula filosa* under the impression that it was a Sea Pen, an error first corrected by Pallas eight years later. This species infests the sun-fish — *Orthagoriscus* — as well as the sword-fish — *Xiphias* — and the double host has suggested to some investigators that the name "filosa" covered an assemblage of ill-described Penellas rather than characterizing a distinct species. This view, however, does not seem well taken. With respect to the forms usually called by the name "filosa," material from either the sun-fish or the sword-fish presents no constant pecularities by which the source may be determined. My immature specimens were taken from sword-fish.

The adult *Penella filosa* frequently attains a length of 130 mm. and may be over 20 cm. long. Of this, almost one half, the round "head" and slender cylindrical "præ-thorax," is buried in the tissues of the host. Only the thicker "post-thorax" and brush-like abdomen trail free in the sea-water, furnishing a locus for a dense growth of hydroids and occasionally barnacles, *Conchoderma*. The abdomen is less than one third of the exposed part of the animal and owes its brush-like appearance to from twenty to thirty pairs of lateral appendages each of which is subdivided into numerous thread-like branches. The trailing egg-sacs are filiform and not infrequently measure 8 or 10 cm. in length. The "head" retains minute, elongate, first, and stouter, chelate, second antennæ on the dorsal surface. Mouth parts are absent and the ventral and anterior surfaces of the "head" are tuberculated. Behind the "head" the præthorax bears four pairs of minute limbs and forward from the anterior pair of these a stout, blunt "horn" projects on either side. This arrangement of horns, however, is not invariable and out of thirty specimens at my disposal from *Orthagoriscus mola* I found nine with an additional dorsal horn. A similar variation in the number of the horns was noted in *Penella varians* by its describers (Steenstrup & Lütken, '61) and parallel conditions are frequently met among parasitic Copepoda which attach to their hosts by analogous structures. A typical specimen of the Lernæenicus of the Menhaden for example — *L. radiatus* — has five simple horns; but specimens are commonly taken which possess a greater or lesser number of these *haftorgane* and not infrequently they are forked or are rough with small branches.

The immature *Penella* in the collection loaned me were five in number. To these a young *Penella filosa* taken from a swordfish brought into Woods Hole has been added, so that my series included six specimens. The youngest individual (*A*) (Fig. 1), had a distinct cylindrical cephalothorax, highly convex, with a double-lensed median eye near the anterior end. Beyond the border of the cephalothorax the three-jointed (?), hamate second antennæ projected. This cephalothorax was flecked with considerable black pigment. The filiform thorax extended to a length thirty-two times that of the cephalothorax. It was not segmented but ringed as in the adult with indistinct circular constrictions near the hinder end. The abdomen was filiform, bore along its sides twenty-two pairs of short unbranched lateral appendages and terminated with a deep notch. On either side of this was a small bisetose appendage. The first pair of antennæ were delicate, two-jointed, and setose. A prominent, urn-shaped rostrum with a reflected, finely serrate border projected from the venter of the cephalothorax and was flanked by reduced maxillæ in the form of minute palps each tipped with two delicate bristles. No traces of mandibles were found. Just behind the rostrum in the mid-line there was a prominent rounded tubercule and on either side of this the two-jointed maxillipeds were situated. Further back, two shallow depressions and back of these two larger shallow pits were visible.

The thoracic limbs appeared to be much like those of an adult *P. filosa* as far as it was possible to examine them. No traces of appendages other than these just mentioned were found. This

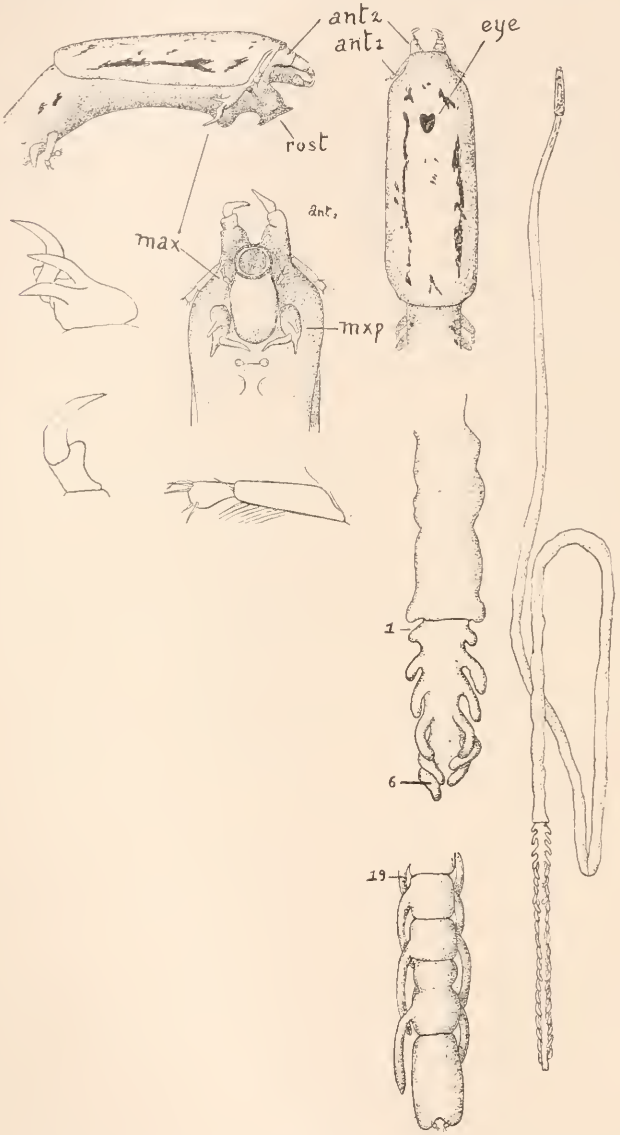

FIG. 1. Youngest larva of *P. filosa*; with three aspects of the carapace, anterior and posterior extremities of the abdomen, maxilliped, first and second antennæ more enlarged.

specimen was 94 mm. long; of which the head measured 2.5 mm., the thorax 80 mm., and the abdomen 11.5 mm.

The next older specimen (*B*) shows a marked advance in development over "*A*" (Fig. 2). The cephalothorax and thorax are no longer distinctly demarked from one another and the sides of the anterior portion of the former region are inflated so that a "head" is differentiated. On the dorsal surface, in the neighborhood of the antennæ, a trace of the borders of the cephalo-thorax still persist. In the region of the anterior pair of thoracic limbs, behind the newly-formed "head" a pair of blunt horns have made their appearance. The median eye was not noted in this individual. The antennæ and maxillipeds are much the same as in stage "*A*," except that the second antennæ are now more chelate. The rostrum is distinctly shifted toward the anterior border of the head and the median tubercule is now reinforced by a conical tubercule on either side of the rostrum. Whether this pair of tubercules replace the maxillæ or not is uncertain. These appendages were not observed in the specimen under consideration, but the minute palps to which they are reduced were doubtfully traced in the next older specimen mesad from the lateral tubercules. The abdominal appendages are uniramous as before. The length of "*B*" was 130.5 mm. The "head" measured 2.5 mm. long by 1.2 mm. wide, the thorax was 120 mm. long, the abdomen 8 mm. long.

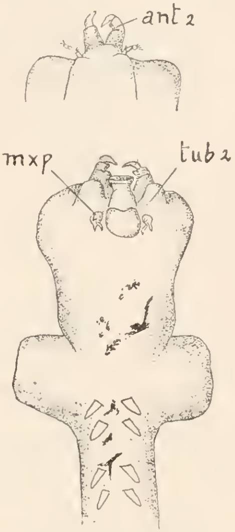

FIG. 2. Second oldest larva "B" of *P. filosa*; dorsal and ventral aspects of the head.

The third specimen (*C*) differed from "*B*" in the still more inflated "head" and in the replacement of the maxillipeds by a pair of tubercules, so that the ventro-anterior face of the "head" now presented five tubercules, one median and four paired. The rostrum of this stage pointed in a line with the axis of the "head" and its tip together with the tips of the anterior pair of lateral tubercules were visible from above. The dimensions of

“*C*” were; head 2.5 mm., thorax 132 mm., abdomen 12 mm. The “head” was 1.8 mm. broad at the widest; the horns spread 2 mm.

In the fourth member of my series “*D*” (Fig. 3) the chief change is the further progress of the shifting upward of the anterior face of the “head.” The tips of the rostrum and anteriormost tubercules are visible from above as before. The antennæ have migrated to the upper surface of the “head” and the apices of the second pair are distant from the anterior border. These antennæ are adult in form. A few of the abdominal appendages consisted of a main ramus and two minute accessory rami, but the majority of this series of appendages had only a single accessory branch and the first five or six pairs were uniramous. The “head” of this specimen measured 3.5 mm., the body 130 mm., the abdomen, 16.5 mm.

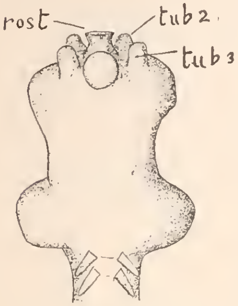

Fig. 3. Fourth larva “D” of *P. filosa*; ventral aspect of the head.

Of the two remaining specimens of the series; “E,” the Woods Hole example, closely resembled “F” (Fig. 4), the last of the primary group. The “head” is now broad anteriorly, but does not as yet approach the rounded form characteristic of the “head” of the adult *P. filosa*. In “E” the antennæ were nearer the anterior margin of the “head” than was the case with “D,” but were not visible from below. A trace of the cephalothoracic border is still present in this anterior dorsal region. The rostrum has disappeared and the venter of the anterior part of the “head” is covered with small tubercles, amid which the tubercules already introduced are lost. In specimen “E” the median one of the older series could just be identified. This specimen also exhibited faint traces of the median eye and the condition of its abdominal appendages was similar to that existing in “D,” the six anterior pairs being unbranched, the remaining pairs having a longer outer and a shorter inner ramus. The rami are longer than the rami of “D's” appendages, however. All the abdominal appendages of “F” on the other hand had two accessory branches and so further approached the complexly-parted appendages of the adult. The dimensions of "E" were; head 3 mm., thorax 150 mm., abdomen 9 mm. The head was 2 mm. wide and the horns spread 3.5 mm. The total length of "F" was 133 mm.

Although fundamentally similar, in several points the development shown by this series differs from the only previous description of undoubted *Penella* larvæ which I have been able to find, that recorded for *P. varians* by Steenstrup and Lütken ('61). This earlier account starts with a larva (Fig. 5) unquestionably much more immature than the youngest form in my series.

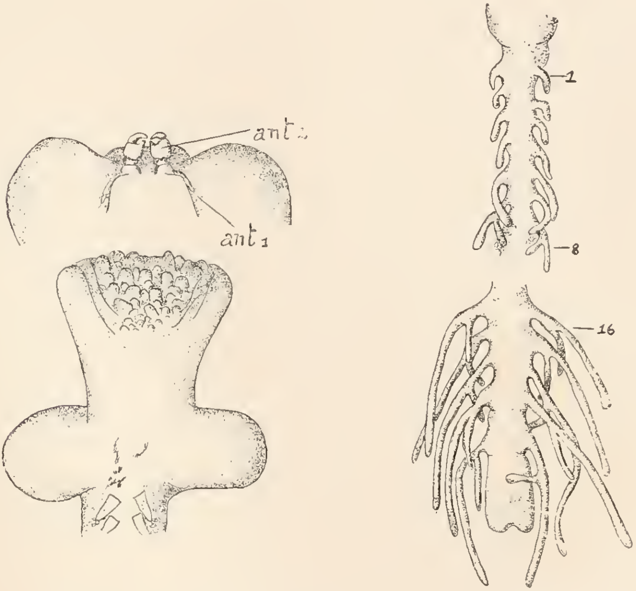

Fig. 4. Head of most mature larva of *P. filosa*; "F". Anterior and posterior extremities of the abdomen of larva "E".

This individual seems to have been about one centimeter in length, was filiform with a highly-convex cephalothorax, succeeded by three short thoracic segments, a long thoracic segment and a short, segmented (?) abdomen. This latter region lacked lateral appendages. The relative proportions of cephalothorax, thorax, and abdomen were — as shown in the figures — about 1′ 4′ 1′;

the posterior or "genital" thoracic segment being ten times as long as the three anterior rings together. The length of the thorax here shown proportionate to the cephalothorax and abdomen is in marked contrast to the excessively elongate thorax of the *P. filosa* larva "A," where the relative lengths of the three body regions are as 1′ 32′ 5′. However, the unlikeness should not be wholly referred to the difference in the ages of the two specimens, because in fully-mature specimens of *P. varians* the thorax is proportionally much shorter than with *P. filosa*. The older larvæ of *P. varians* figured by the Danish observers show a gradual approach toward the proportions characteristic of the adult — 1′ 7′ 2′ as figured — although the abdomen remains in all disproportionately long. On the other hand, in the *P. filosa* series the thorax is about twice as long proportionally to the "head" and abdomen as in the adult; these regions only attaining their final dimensional relations later in the course of the development.

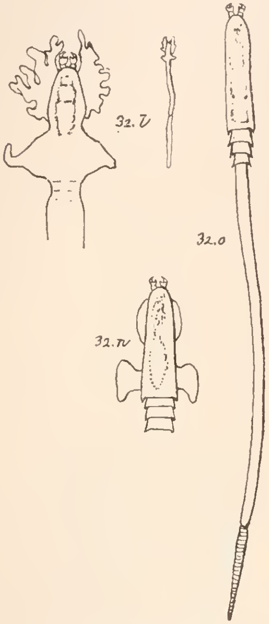

FIG. 5. Youngest, and second and fourth oldest larva of *P varians*. (From Steenstrup and Lütken.)

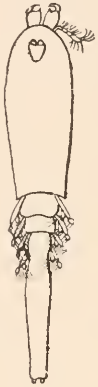

FIG. 6. *Baculus elongatus*. (From Lubbock.)

As with *P. filosa*, the horns of *P. varians* develop as a pair of outgrowths from the sides of the posterior part of the cephalothorax. The metamorphosis of the anterior part of this region of the body to form the "head" is also fundamentally similar in both species. In detail, the development shows noticeable differences. With *P. filosa* no division of the thorax into segments is traceable in my youngest larva, although this has no horns or "head." Later; the sides of the anterior cephalothorax become inflated, early destroying the convexity and distinct borders of the region. The mouthparts are gradually replaced by tubercules, the first of which to appear arise in a definite order. With *P. varians* (Fig. 5) the sides of the anterior region of the cephalothorax beneath the lateral borders of the shield early form two rounded outgrowths, while nearer the posterior end of this part of the body the horns protrude as two longer, pointed outgrowths. This occurs before the separate thoracic segments lose their identity and while the cephalothoracic shield is still distinct in all parts. In an older specimen of this species, the anterior or "head" outgrowths are shown to be broadened and have fimbriated margins. At this time the boundaries between the cephalothorax and thorax and those for the individual thoracic segments are no longer distinguishable while the borders of the cephalothoracic shield are still recognizable anteriorly and the mouthparts are intact. The later metamorphosis is not known.

The rostrum, mouthparts and antennæ of the *P. varians* larvæ seem to have been very like those described for my larvæ. The thoracic limbs are figured with the two anterior pairs bi-ramous, the two posterior pairs uni-ramous. No account is given of the changes which the cephalothoracic appendages undergo, nor of the development of the lateral appendages of the abdomen. It is clear that the latter were not present in any of the larvæ described and the implication is that their development is markedly retarded, although no definite statement is made with regard to this point. It is not improbable that in this species the adult shape of body and "head" may be attained before the abdominal appendages are developed. Such would appear to be the case with another species of *Penella* at least, *P. exocœti*, for Steenstrup and Lütken ('61) figure specimens which have a "head" of almost adult form but lack abdominal appendages. The sexually mature *P. exocœti* evidently possesses abdominal appendages that are subdivided into several branches. The relative development of abdominal appendages compared with the "head" in the oldest specimen of the *P. filosa* series — "F" — suggests the possibility of a somewhat similar phenomenon here, to the extent that individuals may occur with the general appearance of an adult, but with less complexly cleft appendages. Such approaches in development to an adult bodily form before the abdominal structures have fully shaped themselves deserve notice, if only from the standpoint of the systematist. They may readily become not unimportant sources of confusion.

Moreover with *P. varians* the abdominal appendages are variable even in the adult animal, Steenstrup and Lütken recording them as bifid for several mature individuals (p. 411) although they are typically uniramous. With *P. filosa* the abdominal appendages of all the specimens of mature form that I have had opportunity to examine, were complexly subdivided into filamentous branches. The occasional presence of one or more simple or few-parted appendages at the anterior end of the series was the only variation found. This occurred in two out of seventy-nine specimens from *Orthagoriscus mola* in a collection loaned me by the Museum of Comparative Zoölogy. At least one of these was sexually mature and bearing egg-sacs and the same was true for a single specimen out of fifteen examples from the swordfish, which individual had three of the anteriormost abdominal appendages tri-ramous only. The number of the abdominal appendages is fairly constant in *Penella filosa;* varying from 21 to 30 pairs. Twenty-two or twenty-five pairs are the commonest. Of my larvæ: "A" had 22 pairs, "B" 21, "C" 23, "D" 21, "E" 20 and "F" 22.

On comparing the youngest *Penella* larva known, the immature *P. varians* figured by Steenstrup and Lütken, with that stage of the ontogeny of *Lernæa branchialis* at which fertilization and attachment to the final host takes place — the "begattungsstadium" of Claus' ('68) description — or to the corresponding phases of other lernæoid copepoda, the resemblance in general structure is striking. And it appears probable, therefore, that the larva of *Penella* at the time when it attaches to the host has a form not unlike this "mating-stage" of the *Lernæa*. That is, it will closely resemble the youngest stage of the *Penella varians* series with the exception that the posterior or genital segment of the thorax will not be as disproportionately long in comparison to the rest of the body. Very probably this segment will not be sharply distinguished from the abdomen, as in the case in *Lernæa* of this age. A number of years ago Wierzejski ('77) described certain larval Copepods from the gills of *Loligo*, *Sepia* and *Eledone* at Triest and suggested that they might be the mating-stage and younger larvæ of *Penella varians*. His opinion was based on the characters exhibited by the appendages. As figured, however, these structures do not appear to be at all conclusive with regard to this hypothesis and in general form the larvæ certainly do not at all correspond to what would be inferred for the mating-stage of any *Penella*. Two minute immature Copepods have been recorded which seem to fill the requirements better. These were described under the names *Baculus elongatus* (Lubbock, '60) and *Hessella cylindrica* (Brady, '83). The former was collected in the Gulf of Guinea, the latter off Zamboanga in the Sulu Archipelago. Although not wholly alike in all structural details, they agree in possession of a filiform shape, cylindrical cephalothorax with projecting second antennæ, short segmented thorax, with four pairs of limbs, and an elongate hind-body devoid of lateral appendages and evidently representing a fusion of the posterior part of the thorax with the abdomen. Lütken in 1892 strongly urged that *Baculus elongatus* be regarded as a larva of *Penella*. He points out that *Baculus elongatus* is a pelagic form, in harmony with the fact that the genus *Penella* infests "fishes or whales with a more or less marked pelagic habit (*Xiphias*, *Coryphæna*, *Exocœtus*, *Diodon*, *Mola*, *Pterophryne*, *Hyperoödon*, *Balænoptera*)." The filiform shape of these larvæ unquestionably suggests *Penella* and the argument from habitat is significant. But, nevertheless, it must be admitted that on a basis of present knowledge of the forms a definite determination is not possible. We can only say that *Baculus* or *Hessella* are almost certainly the young of lernænoid copepods, possibly of *Penellas*. However this may be, these larvæ are helpful in constructing a picture of the hypothetical mating-stage phase of *Penella's* metamorphosis.

Attached to the host while possessing such a *Baculus*-like form, probably at first by the antennæ, then later burrowing into the tissues, the retrogressive metamorphosis begins. An elongation of the posterior thoracic segment brings about a condition in which the young *Penella* will parallel the earliest described stage of *P. varians.* Then the adult form is gradually developed. The anterior part of the cephalothorax moulds itself into the "head," the horns protrude from the posterior part of this region, the thoracic segments coalesce, the anterior ones remaining undeveloped, the posterior elongating to form the major part of the body, while the abdomen develops the characteristic lateral appendages.

As regards the metamorphosis prior to the attainment of the mating-stage form, we only know that the young *Penellas* almost unquestionably hatch as nauplii. The analogies presented by the development of *Lernæa* and allied copepods (Scott, '01, Claus, '68) suggests the following cycle in the period between the nauplius and the completed, fertilized, mating-stage larva. The nauplius probably metamorphoses to a cyclops-like form, the so-called "*Chalimus*" stage, and in this phase the young attach themselves to some host. Typically among parasitic copepods, the attachment in this chalimus stage in made by a frontal filament. While thus fixed on the host, the mating stage is reached by successive moults. At this point in the ontogeny of *Lernæa* the male and females copulate and the latter detach themselves and for a short period return to a pelagic life. Then they settle down upon the final host, which happens to be a different kind of fish from the one they infested during the earlier part of their life. Other parasitic copepods pass through a similar life cycle on a single species of animal as host. Of course we have no clue to which of these groups *Penella* belongs, but its Lernænoid affinities suggest a metamorphosis with the double host. Nevertheless, this possibility should in no way discourage the most careful scrutiny of freshly captured fishes that are infested with *Penellas* or are species which serve as hosts for this genus, if opportunities present themselves. The metamorphosis must run a course similar to that in *Lernæa*, and if only a newly-attached mating-stage larva could be identified and described, it would go far toward the unravelling of the life-history of these highly-modified copepods.

Similarly, the discovery of the males for this genus is much to be desired. Steenstrup and Lütken ('61) record finding near the egg-sacs of a *Penella exocœti* two almost microscopic animals which reminded them of the "pigmy males of other lernæan forms." They figure one of these with the suggestion that it may be the vainly sought male. The specimens could not be removed for study. It is very doubtful whether this is a male of *Penella*. It would seem that the male of this genus ought rather to resemble the male of *Lernæa* and its form will be cyclopoid much like that of the chalimus stage of the females. As with *Lernæa* also, it is unlikely that the males remain attached to the females after fertilization has taken place, but continue on the host which has served them for their earlier development, while the females become free swimming after fertilization and settle down on another animal of the same or another species from the first host.

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THE REARING OF SERPULID LARVÆ WITH NOTES ON THE BEHAVIOR OF THE YOUNG ANIMALS.[1]

CHARLES ZELENY.

The Method of Rearing.

The complete success of an attempt to rear serpulid larvæ[2] has prompted the present description of the method, in view of the fact that other workers have been unsuccessful in the same field.[3] It is hoped that the method may be found valuable not only for serpulid larvæ, but also for the great majority of forms which do not allow a direct current of water to be passed in and out of the dish containing them.

The eggs after fertilization were placed in large glass beakers and the water was changed several times during the first day. They were then allowed to develop into free swimming trochophores which collected in great numbers at the surface of the water during the second day. A few of these were removed with a pipette and placed in a "battery jar" containing fresh sea water obtained in the open harbor at rising tide on a bright afternoon. Not more than a few hundred larvæ were put into any one battery jar. The jars were covered with glass plates to keep out the dust, and were placed in such a position that the rising sun shone on them for about an hour just after sunrise each morning. Immediately after the sun bath they were cooled off in buckets of cold spring water and were then placed for the rest of the day in shallow basins of the same cold water. In this way the proper conditions for the development of the microscopic algæ and other organisms which serve as the food of the larvæ were furnished without at the same time heating the water to such an extent as to kill the larvæ themselves.

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[1] Contributions from the Zoölogical Laboratory of Indiana University, No. 65.

[2] *Hydroides dianthus.*

[3] The work described was done at the Cold Spring Harbor Biological Station during the summer of 1902, while the writer was holding the John D. Jones Scholarship at that place. My best thanks are due to Professor C. B. Davenport, the director of the station, for his kindness in supplying me with every possible facility while at the laboratory. Some of the notes refer to work done at the Naples Zoölogical Station during the winter of 1902–3.

Under the above treatment the larvæ flourished and successfully went through the transformation from the free-swimming to the sedentary condition. They settled down on the sides of the vessels in great numbers about fourteen days after fertilization and observations could readily be made upon them with a horizontal microscope. The examination of the larvæ can undoubtedly be facilitated by the suspension of glass slides in the water at the places where the free-swimming larvæ are about to attach themselves. The slides with the attached larvæ can then be transferred to shallow dishes of sea water and examined with the microscope in its ordinary upright position.

A study of the development of the opercula in the serpulids constituted my main object in rearing the larvæ and a description of this feature is incorporated in a paper now in press.[1] A few incidental observations on the behavior of the young animals are given in the following notes :

Behavior of the Young Serpulids.[2]

The Free Swimming Larvæ. — As soon as the pre-oral band of cilia is well developed the young larvæ swim toward the surface of the water and collect there in great numbers, especially at the edges near the glass sides of the jar. They are always more crowded in certain regions than in others, but the exact relation of this crowding to a phototactic response was not made out because of the complex relations due to refraction and reflection of the light within the jar. The greatest crowding was usually on the side of the jar facing the window and on the side directly away from the window. At the latter place the collection of larvæ may have been due to a secondary reflection of the light within the jar.[3]

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[1] "Compensatory Regulation." To appear in the *Journal of Experimental Zoölogy*, Vol. II., No. 1.

[2] *Hydroides dianthus* (at Cold Spring Harbor), unless otherwise noted.

[3] A. Giard ('76) in his "Note sur l'embryogénie de la Salmacina Dysteri Huxl." (*Compt. Rend.*, Tome 82, 1876, pp. 233–235, 285–288) says that the free-swimming larvæ of *Salmacina* collect on the sides of the aquarium facing the light while they settle down and form tubes on the side away from the light.

Attachment of the Larvæ. — The period at which attachment of the larvæ should take place is a critical one and death is the usual result under ordinary laboratory conditions. In my experiments at Cold Spring Harbor, however, there were hundreds of survivors in each jar. On account of the frequent change of position of the jars, as well as the complex refractions and reflections within them, I was not able to find out whether the place of attachment of the larvæ has any definite relation to the source of the light. The young Serpulids were found to be fairly uniformly distributed, though there were groupings at several different places. One of these consisted of a large number collected on the glass just below the surface film of the water. These formed a band surrounding the jar, but it is interesting to note that they did not grow as rapidly as those lower down, although fresh sea water was added in each case to raise the level slightly and make up for evaporation. Going down from this band-like zone of greatest frequency the number of individuals decreased until the bottom of the vessel was reached, where again there was a considerable number, especially in the corner between the bottom and the sides of the jar.

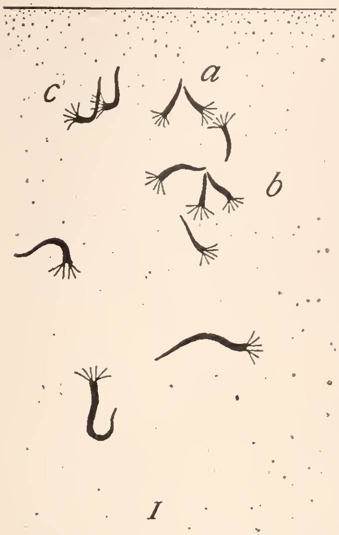

Fig. 1. (× 4.) A group of the young of *Hydroides dianthus* as attached to the side of a battery jar. The level of the water surface is shown. *a* and *b* are groups showing radial divergence of the tubes. *c* shows two tubes which have evidently responded to a lateral stimulus. No definite relation to gravity is shown.

Tube Formation. — The tube when first formed is a very narrow, almost transparent ring of calcareous matter, the body of the short larva extending out of it at both ends. This ring is secreted by the region just back of the free anterior end of the thoracic membrane and as its formation goes on the animal can be seen to extend its thoracic membrane over the anterior edge of the tube in order apparently to smooth the edges and get the material in shape to fit the body. At such a time the body may project a considerable distance from the anterior end of the tube. The tube is deposited quite rapidly. In the case shown in Fig. 2 the amount of growth in the course of twenty hours is given. This is equal to .29 mm. or .35 mm. per day.

I tried to discover some regularity in the arrangement of the axes of the tubes with respect to gravity, light and food conditions but was able to find no general rule in the matter although some groups seem to be arranged with respect either to maximum food-obtaining ability or with respect to a lateral stimulus of unknown character. Fig. 1 which is reproduced from my notebook gives a small section of a side of one of the jars. Evidently there is no general rule of arrangement though radial divergence of the kind shown at *a* and *b* (Fig. 1) may be explained on a utilitarian basis as a spreading out from a central point in order to obtain more feeding room. The arrangement at *c* however does not come under this head but must be considered as a very definite response to a lateral stimulus. It is hard to conjecture what this stimulus may have been.

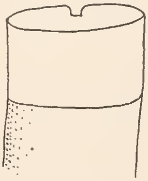

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FIG. 2. (× 38.) Open end of a tube of *Hydroides uncinata.* The unshaded portion represents the amount of addition to the tube in the course of twenty hours.

General Activities of the Young at a Later Stage. — The following observations were made on young individuals during the few days preceding and the few days following the formation of the operculum. Specimens of *Hydroides uncinata* and *H. pectinata* observed at the Naples Zoölogical Station during the winter of 1902–1903 as well as the *H. dianthus* of Cold Spring Harbor were used.

The two eye spots are very prominent at this stage and a sudden shadow such as is produced by the passing of a hand between the animals and the source of light causes the serpulids to contract and withdraw with a jerk into their tubes. The branchiæ are then completely hidden inside of the tube and before the development of the operculum their ends form a barricade a short distance within the opening. After a short period of inaction if there is no new disturbance the fine thread-like ends of the branchiæ begin to appear and wave back and forth around the mouth of the tube as if feeling for signs of danger. In this position the animal usually remains for several minutes often with nothing more than the ends of the two longest branchiæ projecting from the tube. After satisfying itself in this manner the animal pushes itself forward and expands the whole branchial crown with comparative rapidity. After the development of the functional operculum this organ is pushed to one side and the branchial ends are slightly projected around its margin before the thrusting out of the whole branchial circlet takes place (see Fig. 3). The young animals, as well as the adults, are extremely sensitive to the slightest mechanical jar, a very small shock being sufficient to keep them within their tubes for a considerable time. At Naples I was often greatly annoyed while attempting to make drawings of the animals in an extended state to see them jerk back into their tubes at the sound of a band starting to play in the adjacent park. Under such conditions they "sulked" in their tubes until the musical selection was finished.

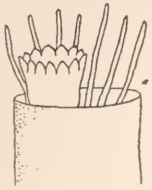

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Fig. 3. (× 38.) *Hydroides pectinata.* The young serpulid has thrust out the ends of its branchiæ preparatory to the expansion of the whole branchial circlet.

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SOME FURTHER EXPERIMENTS ON SELF-FERTILIZATION IN CIONA.

T. H. MORGAN.

My previous experiments with *Ciona intestinalis* had shown that, by adding chloroform, or alcohol, or ammonia to the sea water, self-fertilization could sometimes be effected, although in this species self-fertilization does not usually take place in sea water. The experiments did not show why under normal conditions self-fertilization does not occur. Other results appeared to indicate that the eggs offer a resistance of some kind to the entrance of the spermatozoa of the same individual and that the lack of fertilization is due to the failure of the spermatozoa to enter, and not due to their failing to stimulate the eggs after entering. An analysis of the conditions led me to the conclusion that the inability of the spermatozoa to penetrate eggs of the same individual could not depend on differences in size of the micropyle of the egg that might be correllated, supposedly, with the size of the spermatozoa of the same individual ; also that the failure was not due to the lack of some exciting substance present in eggs of other individuals. I hazarded the guess that the resistance offered by the egg to the entrance of its "own" sperm might be due to some substance contained in the egg, or in its membranes, that brings the spermatozoa of the same individual to rest, and I pointed out that this view could be tested by the following experiment. If the eggs of an individual (A) were removed and allowed to stand in sea water, and if then this water were poured off and the spermatozoa of the same individual (A) were added to the water, they should be so affected that they would fail to fertilize the eggs of another individual (B). I should have made the *proviso*, as subsequent events have shown, that this result would follow, provided the substance that prevents self-fertilization is soluble in sea water. This experiment I have carried out during the past summer in a variety of ways, but have found that the anticipated results did not follow, from which I am led to conclude that the phenomenon is not due to a substance that is soluble.

The failure of this experiment to give positive results showed me that I must start once more at the very beginning, and test more thoroughly the assumption that the sperm of one individual, if in good condition, is capable of fertilizing equally well the eggs of all other individuals; for the difficulty of explaining the results becomes immensely greater if this condition holds absolutely. If it does hold it would mean that the conditions present in an individual are not found in any degree in any other individual. The principal results of this paper deal, therefore, mainly with this question, although, at the same time, I have described some other experiments which gave negative results, because, I think, the results, negative though they be, will be of value in determining the direction of further experiments.

My work was carried out at the marine laboratory of the University of California, situated at present at Coronado Beach, California. I am under many obligations to the university for the privilege of working at the station, and especially to the director, Professor William E. Ritter, and to the resident naturalist, Mr. B. M. Davis. It gives me great pleasure to acknowledge my appreciation of the many courtesies extended to me during my sojourn at the station. Professor Ritter informs me that the species of *Ciona* at Coronado Beach appears to be identical with *Ciona intestinalis* of Europe.

Experiments in Cross-fertilization.

The eggs of an individual were removed and distributed in six dishes, A–A. Similarly the eggs of another individual were taken out and distributed in six dishes, B–B; and, so on, for four other individuals. See Table I. The sperm of A was then removed from the vas deferens and added to A, B, C, D, E, F. In the table the sperm is indicated by the small letters, a–f, used as exponents. The sperm from each of the other five individuals was used in the same way. Thus all the eggs were crossed with all the different kinds of sperm, and self-fertilization was also tried in each case. The self-fertilized lots form a diagonal line across the tables. In only a single case out of the many hundreds of eggs mixed with their own sperm did fertilization occur. It is needless, perhaps, to add that separate scissors, pipettes, etc., were used for each individual. In each experiment there were thirty-six fertilizations made, of which thirty were crosses, and six self-fertilizations. In the eight experiments that I carried out there were therefore 240 cross experiments, which, if not a very extensive series, yet ought to suffice to show the main points. The percentages given in the following tables indicate the proportionate number of eggs in each set that segmented ; the estimated number are approximations only, and not, in all cases, the results of exact counts.

TABLE I.

$\mathbf{A}^a$	A^b 90	A^c 80	A^d 10	A^e 0	A^f 0
B^a 0	$\mathbf{B}^b$	B^c 3	B^d 0	B^e 0	B^f 0
C^a 100	C^b 80	$\mathbf{C}^c$	C^d 100	C^e 75	C^f 0
D^a 90	D^b 75	D^c 80	$\mathbf{D}^d$	D^e 0	D^f 0
E^a 20	E^b 25	E^c 0	E^d 0	$\mathbf{E}^e$	E^f 0
F^a 0	F^b 0	F^c 0	F^d 0	F^e 0	$\mathbf{F}^f$

An analysis of the results shown by this table leads to the following conclusions. The A-eggs were in good condition (or more briefly were "good"), since with "good" sperm 90 per cent. of them were fertilized in one case. The B-eggs were poor, the C-eggs excellent, the D-eggs very good, the E and F-eggs were poor. The a-sperm was excellent, as seen in C^a ; the b, c, d, e-sperm were also excellent ; but the f-sperm was very poor, since not a single egg in any of the sets was fertilized by it. From the results of this table it looks as though good sperm would always fertilize good eggs of any other individual, but there are even here three significant exceptions to this statement. The d-sperm gave poor results with A-eggs (A^d), although other experiments show that both the sperm and the eggs of these two individuals were good. The other cases are those of A^e and D^e, in which the e-sperm failed completely with the A and with the D-eggs, although with the C-eggs it gave 75 per cent. of segmenting eggs.

There was also another point that I wished to test by means of these experiments, namely, whether there is any correspondence between normal and abnormal development of the eggs and the proportion of the eggs that are fertilized. It will be recalled in this connection that Castle found that those cases of *Ciona intestinalis* in which self-fertilization occurs development is abnormal. On the contrary, I found that self-fertilized eggs often give rise to normal embryos. I wished, therefore, to see whether in cross-fertilized eggs there is any relation between the number fertilized and the condition of the embryos. In the following table the kinds of embryos that developed from the eggs given in the preceding table are recorded. The abnormal records generally indicate tadpoles that were bent, or crooked, or otherwise abnormal in shape.

TABLE I.—*Continued.*

$\mathbf{A}^a$	A^b Abn	A^c Abn	A^d Abn	A^e o	A^f o
B^a o	$\mathbf{B}^b$	B^c Abn	B^d o	B^e o	B^f o
C^a Abn	C^b Abn	$\mathbf{C}^c$	C^d Abn	C^e Abn	C^f
D^a Abn	D^b Abn	D^c Abn	$\mathbf{D}^d$	D^e o	D^f o
E^a Abn	E^b Abn	E^c	E^d	$\mathbf{E}^e$	E^f o
F^a o	F^b o	F^c o	F^d o	F^e o	$\mathbf{F}^f$ o

The table shows that all of the tadpoles were abnormal, despite the fact that a large percentage of the eggs was fertilized in several cases. The next table gives the results of a second experiment.

TABLE II.

$\mathbf{A}^a$	A^b 10^b	A^c 40	A^d 50	A^e 0	A^f 40
B^a 30	$\mathbf{B}^b$	B^c 90	B^d 80	B^e 1	B^f 25
C^a 90	C^b 95	$\mathbf{C}^c$	C^d 99	C^e 100	C^f 100
D^a 90	D^b 90	D^c 80	$\mathbf{D}^d$	D^e 1	D^f 99
E^a 0	E^b 40	E^c 25	E^d 50	$\mathbf{E}^e$	E^f 0
F^a 1	F^b 40	F^c 80	F^d 15	F^e 0	$\mathbf{F}^f$

The A and the E-eggs were only moderately good, the B, C, D, and F-eggs were very good. The sperm in all the sets gave very good results with at least some of the eggs. The exceptional cases found in this series were as follows. The a-sperm did poorly with B, very poorly with F, and gave nothing with E, although with C and with D 90 per cent. of the eggs were fertilized. The b-sperm also gave varying results, although the extremes were not so marked; and this holds to even a less degree with the c-sperm. The c-sperm gave exceptionally good results with the F-eggs. There is nothing calling for comment in the behavior of the d-sperm; while the e-sperm was surprisingly deficient with the B and the D-eggs, although excellent with C. The f-sperm gave excellent results with the C and the D-eggs, but was less good with B. The later development of the eggs in this series was not observed.

TABLE III.

$\mathbf{A}^a$	A^b 80	A^c 80	A^d 1	A^e 0	A^f 0
B^a 100	$\mathbf{B}^b$	B^c 100	B^d 10	B^e 2	B^f 1
C^a 100	C^b 100	$\mathbf{C}^c$	C^d 20	C^e 25	C^f 1
D^a 100	D^b 100	D^c 50	$\mathbf{D}^d$	D^e 5	D^f 10 (1 in 10)
E^a 20	E^b 10	E^c 10	E^d 2	$\mathbf{E}^e$	E^f 0
F^a 20	F^b 70	F^c 15	F^d 1	F^e 0	$\mathbf{F}^f$

The A, B, C, D-eggs appear to have been excellent; the E-eggs poor, and the F-eggs probably good. The a and the b-sperm appear to have been good, and the latter, in the case of F, gave unexpectedly good results. The c-sperm was also good, but gave with D only 50 per cent. of segmenting eggs. The d, e, f- sperm was poor.

The embryos (tadpoles) were examined after 24 hours with the results recorded in the following table:

TABLE III.—*Continued.*

$\mathbf{A}^a$	A^b Abn.	A^c Abn.	A^d Abn.	A^e One abn.	A^f 0
B^a Abn.	$\mathbf{B}^b$	B^c Nearly norm.	B^d Abn.	B^e Abn.	B^f One norm.
C^a Norm.	C^b Norm.	$\mathbf{C}^c$	C^d Most abn. One norm.	C^e Most norm.	C^f One norm.
D^a Most abn.	D^b Most abn.	D^c Abn.	$\mathbf{D}^d$	D^e Abn.	D^f Abn.
E^a Abn.	E^b Abn.	E^c Abn.	E^d	$\mathbf{E}^e$	E^f Abn.
F^a Abn.	F^b Abn.	F^c Abn.	F^d 0	F^e 0	$\mathbf{F}^f$

Although A^b and A^c gave 80 per cent. of cases of fertilization they produced only abnormal tadpoles. B^a gave also only abnormal tadpoles, while B^c gave nearly normal tadpoles. Although only one egg in B^f was fertilized it produced a normal tadpole. The C-eggs gave even more curious results. The C^a and C^b-eggs gave exceptionally normal and active tadpoles; the C^d-eggs were abnormal (excepting for one individual); most of the C^e were normal, while the one egg fertilized in C^f was normal. The D^a and D^b-eggs gave very abnormal tadpoles, but some of them were normal, while D^c and D^e and D^f were abnormal.

All of the E and the F-eggs gave abnormal tadpoles. These results show very clearly that there is no necessary relation between the percentage of eggs fertilized and the normal or abnormal development that takes place, although in general good eggs are more likely to produce normal tadpoles, and poor eggs abnormal ones.

Table IV.

A^a	A^b 15	A^c —	A^d —	A^e —	A^f —
B^a 30	**B^b**	B^c 35	B^d 5	B^e 0	B^f 0
C^a 5	C^b 0	**C^c** 1 in 10	C^d 0	C^e 0	C^f 0
D^a 20	D^b 10	D^c 3	**D^d**	D^e 8	D^f 0
E^a 100	E^b 100	E^c 95	E^d 100	**E^e**	E^f 0
F^a 75	F^b 1 in 10	F^c 4 in 26	F^d 1 in 50	F^e 0	**F^f**

In this series the A-eggs were not distributed through a mistake except in two cases. The B and the D-eggs were poor; the C-eggs very poor; the E-eggs were excellent; and the F-eggs seem to have been good. The a-sperm gave the best results, yet failed to give high percentages with the poorer eggs; and the b-sperm behaved similarly, but did less well with F. The c and the d-sperm were excellent with the e-eggs only, while the e and the f-sperm were poor in all cases, showing, in the case of the E-eggs, which were excellent, that the latter could not fertilize eggs as good as these.

Table V.

A^a	A^b 0	A^c 0	A^d 0	A^e 0	A^f 100
B^a 100	**B^b**	B^c 5	B^d 0	B^e 0	B^f 80
C^a 30	C^b 10	**C^c**	C^d 0	C^e 0	C^f 80
D^a 0	D^b 2	D^c 0	**D^d**	D^e 0	D^f 14
E^a 5	E^b 25	E^c 5	E^d 0	**E^e**	E^f 30
F^a 75	F^b 20	F^c 2	F^d 0	F^e 0	**F^f**

This experiment gave some curious results. The A-eggs must have been excellent to judge by A^f, yet gave no results in other cases. The B-eggs must have been excellent, but gave good results only with the a and the f-sperm. The C-eggs gave similar results, but few eggs segmented with the a-sperm. The D-eggs must have been poor giving only 14 per cent. with the f-sperm. The E-eggs also gave poor results,[1] and the F-eggs were well fertilized only with the a-sperm. The development of the eggs as shown by the following table gave some unexpected results when considered in the light of the number fertilized :

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[1] The E-eggs were polyspermic.

TABLE V.—*Continued.*

$\mathbf{A}^a$	A^b Norm.	A^c Abn.	A^d 0	A^e 0	A^f Norm.
B^a Abn.	$\mathbf{B}^b$	B^c Norm.	B^d 0	B^e 0	B^f Norm.
C^a Norm.	C^b Abn.	$\mathbf{C}^c$	C^d 0	C^e 0	C^f Abn.
D^a ?	D^b Abn.	D^c 0	$\mathbf{D}^d$	D^e 0	D^f Abn.
E^a Abn.	E^b Abn.	E^c Norm.	E^d 0	$\mathbf{E}^e$	E^f Abn.
F^a Norm.	F^b Norm.	F^c 0	F^d 0	F^e 0	$\mathbf{F}^f$

The B^a-tadpoles were abnormal although 100 per cent. of the eggs were fertilized ; while the B^c-tadpoles were exceptionally normal. Although the c-sperm was very poor, the B^c and the E^c-tadpoles were exceptionally active and normal. The E^f-tadpoles were only somewhat abnormal, although the eggs appear to have been poor. The F^a and the F^b tadpoles were excellent, although only 20 per cent. of the eggs divided in the latter case.

TABLE VI.

$\mathbf{A}^a$	A^b 0	A^c 0	A^d 20	A^e 0	A^f 0
B^a 100	$\mathbf{B}^b$	B^c 25	B^d 75	B^e 65	B^f 95
C^a 90	C^b 90	$\mathbf{C}^c$	C^d 90	C^e 90	C^f 50
D^a 0	D^b 2	D^c 0	$\mathbf{D}^d$	D^e 20	D^f 20
E^a 20	E^b 95	E^c 40	E^d 100	$\mathbf{E}^e$	E^f 80
F^a 1	F^b 95	F^c 30	F^d 100	F^e 40	$\mathbf{F}^f$

The most striking results shown by this table are that while the F-eggs were excellent and the a-sperm also, yet F^a gave only one per cent. of segmenting eggs. The e and f-sperm gave much better results with D-eggs, than did any other sperm with these eggs, although some of the other sperm appears to have been excellent.

TABLE VII.

$\mathbf{A}^a$	A^b 0	A^c 0	A^d 8	A^e 0	A^f 0
B^a 15	$\mathbf{B}^b$	B^c 5	B^d 5	B^e 0	B^f 0
C^a 70	C^b 80	$\mathbf{C}^c$	C^d 95	C^e 80	C^f 20
D^a 12	D^b 1	D^c 8	$\mathbf{D}^d$	D^e 6	D^f 0
E^a 50	E^b 70	E^c 50	E^d 95	$\mathbf{E}^e$	E^f 0
F^a 80	F^b 60	F^c 30	F^d 95	F^e 75	$\mathbf{F}^f$

In this table also there are several cases where the sperm was less capable with some eggs than with others, even when other cases showed that the eggs were equally good. The differences are not so striking as in some of the other series.

Table VIII.

A^a	A^b 20	A^c 85	A^d 75	A^e 0	A^f 35
B^a 0	**B^b**	B^c 20	B^d 25	B^e 0	B^f 0
C^a 0	C^b 0	**C^c**	C^d 8	C^e 0	C^f 0
D^a 8	D^b 50	D^c 90	**D^d**	D^e 0	D^f 7
E^a 0	E^b 8	E^c 80	E^d 50	**E^e**	E^f 0
F^a 0	F^b 3	F^c 8	F^d 15	F^e 0	**F^f**

With the exception of c- and of d-sperm the sperm appears to have been very poor in this series. The B, C and F-eggs were also poor. It is noticeable that the results appear to depend in this case more on the condition of the sperm than of the eggs.

In my previous paper five tables,[1] similar to these, were given for the *Ciona* found at Woods Hole. The incompatability was less marked, although some cases, apparently of this sort, were found. In general, however, the eggs crossed much more readily than did the California form. Since the individuals at Woods Hole were collected over a wider area than were those at Coronado Beach, all of which grew on the same float, the chance is greater that they may have had a different parentage. Whether the closer descent may account for the greater incompatability in the Coronado individuals can not be stated, but the facts are suggestive.

Discussion of Experiments.

The most obvious question that suggests itself in those cases where few eggs were fertilized is whether if more sperm was added more eggs would segment. In answer to this I should point out that an approximately equivalent amount of the same solution containing sperm was added in each vertical series. Another experiment made to test this point showed that the amount added was many times greater than that necessary to fertilize the eggs when a good combination is made. Thus 3, 6,

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[1] A few errors in my former tables may be corrected here. Thus in all the tables A^e appears twice, and E^a also. In experiment XIV. the first A^e gave 0 per cent., the second 100. This is due no doubt to the failure to add sperm to the first lot, which might easily occur. This is apparently also the case in experiment XV. In experiment XVI. the first E^a gave only 5 per cent., the last 70. This difference I cannot now account for. B^c in this table should be B^d, and C should be C^e.

12 and 24 drops of a solution containing the sperm was added to equivalent numbers of eggs, and gave 90, 90, 90, 100 per cent. of eggs fertilized. In another case 3, 6, 12 and 24 drops gave 6, 50, 90, 90 per cent. In the latter case three drops were not enough; six not quite enough; but 12 and 24 sufficed. Now since in all the other experiments more than 24 drops were used the outcome must depend in only a small degree on the quantity of sperm, and the results represent, therefore, approximately the proportion of eggs and sperm capable of uniting.

The tables show that the sperm is generally "at fault," when fertilization does not occur, the eggs being in nearly all cases capable of fertilization when good sperm is used, although, as pointed out, some striking exceptions occur. Thus poor sperm will sometimes fertilize more eggs of one set than of another, but the better the sperm the more eggs it will fertilize as a rule.

It is clear from the tables that it is erroneous to suppose that the sperm will fertilize equally the eggs of all other individuals. All degrees of sterility are met with in cases where other experiments with the same sperm fully succeed. Therefore the problem is not so sharply defined as appeared to be the case before these experiments were made.

Experiments to Determine the Nature of the Influences Preventing Self-Fertilization.

As stated above these experiments were undertaken in the hope of finding out whether there is a soluble substance in the eggs or body tissues of an individual that so affects its own sperm that it is rendered incapable of fertilizing its own eggs. If this is the case it would seem probable that the sperm might be affected by extracts of its "own" tissues, so that it might no longer be capable of cross-fertilization, if eggs of another individual were added to the extract containing the sperm.

The experiments to test this are not so simple as may appear on first thought, since check experiments must be carried out in order to see whether the same action may not result when extracts of the body of another individual are used. In fact it would have been very easy to make a serious blunder had not this precaution been taken.

The converse experiment should also be carried out at the same time; namely, that in which the sperm of A is put into the extract of B in order to see if it might not be made active so that it would then fertilize its own eggs. In my previous paper I have described some experiments of this kind. As a check to this the a-sperm should be put into the B-extract and then later the B-eggs added to show whether the extract itself may not interfere with the fertilization. The complete experiment is indicated in the following table:

A-sperm.	B-extract.	Add A-eggs	(1)
		Add B-eggs	(2)
	A-extract.	Add A-eggs	(3)
		Add B-eggs	(4)
B-sperm.	B-extract.	Add A-eggs	(5)
		Add B-eggs	(6)
	A-extract.	Add A-eggs	(7)
		Add B-eggs	(8)

Experiment 1. — In one experiment in which an extract of the ovary of the individuals was used none of the eggs segmented showing that even the extract of another individual prevents the cross-fertilization from taking place. A test experiment with these same eggs and sperm, brought together in sea water, showed that A-eggs with B-sperm gave only 20 per cent. of cases of segmentation, and B-eggs with A-sperm gave no cases of fertilization. It is clear, therefore, that the eggs and sperm were not in very good condition. When self-fertilized in sea-water neither the A-eggs or the B-eggs segmented.

Experiment 2. — In another experiment the eggs and the ovary of one individual (A) were soaked in sea water for one hour. The same was done with the eggs and the ovary of another individual (B). The sperm of A was added to the A-extract and the B-sperm to the B-extract for half an hour. Then the B-eggs were placed with the A-sperm that had been in the A-extract, and the A-eggs with the B-extract and B-sperm. None of the eggs divided. In this same experiment the B-sperm was put into the extract of the ovary of A, and later the A-eggs were added; similarly for the A-sperm. No eggs were cross-fertilized, showing as in the preceding case that the extract itself had interfered with cross-fertilization (provided the eggs were good which was not tested).

Experiment 3. — In this experiment the eggs (instead of the sperm) were soaked for a short time in the extract of the ovary of another individual in order to see if they might not acquire the power to become fertilized by the sperm if this were then added.

The A-eggs were soaked in the extract of the ovary of B about 15 minutes. The A-sperm was added to sea water, and left for 5, 10 and 15 minutes. Then the A-eggs were added to the A-sperm solutions. No eggs segmented, but neither did the eggs in the check experiment in sea water, showing that the eggs or the sperm were poor, or else incompatible. In the check experiment (B-eggs in extract of A-ovary), however, 100 per cent. of the B-eggs were fertilized by the A-sperm. This also happened in the cross in sea water. Here the A-extract did not prevent the B-eggs from being cross-fertilized by A-sperm. The solution must have been sufficiently dilute for fertilization to take place.

Experiment 4. — In this case the mantle was removed, and the body wall slightly cut so that the heart protruded, which was cut open and the blood collected. The eggs of another individual were then put into the blood. There is here a possible source of contamination, since some of the follicles of the testis may be broken, and allow some of the sperm to get into the blood. The results of one experiment of this sort were as follows :

			In Blood.	Diluted Later.
(1)	A-eggs	A-blood B-sperm	0	40 %
(2)	B-eggs	A-blood A-sperm	0	70
(3)	A-eggs	B-blood B-sperm	0	0
(4)	B-eggs	B-blood A-sperm	0	0

The check crosses carried out in sea water gave for the A-eggs (B-sperm) 90 per cent. of cases of segmentation, and for the B-eggs (A-sperm) 50 per cent. The first two combinations (1) and (2) of the table, gave no results in the blood itself, but when the blood was diluted they gave nearly the same percentages as in sea water. In the second cases the dilution was probably insufficient. Thus it is shown that the undiluted blood interferes with the fertilization, but on the other hand the diluted blood of the same individual does not affect the sperm so that it fails to cross-fertilize.

Experiment 5. — The eggs were put into the blood of another individual for twenty minutes, and then removed to sea water, and their own sperm added. No self-fertilization followed, although direct cross-fertilization gave 70 per cent. for the B-eggs, and 100 per cent. for the A-eggs, showing that both eggs and and sperm were good.

Experiment 6. — In order to test whether the blood itself may not sometimes become contaminated with its own sperm, one portion of the blood of A was diluted with sea water, and B-eggs were then put into it. In one case a few eggs segmented, showing that contamination may occur. Heating the blood would, of course, kill the sperm, but this would open the experiment to the objection that the composition of the blood might be so affected that the postulated soluble substance is destroyed. Since most of the experiments gave negative results the contamination of the blood, if it occurred, would not vitiate such results; but if the results had been positive this possibility would havehad to be carefully reckoned with.

EFFECTS OF SHAKING.

It seems not improbable that the covering of test-cells might be the immediate cause of the lack of power to self-fertilize. Therefore I tried the effect of shaking them off, and adding sperm of the same individual to the denuded eggs.

Experiment 7. — In this case only one egg out of 200 segmented, while 400 of the same eggs unshaken showed no segmentation.

Experiment 8. — In another case about 20 per cent. of the shaken eggs segmented, but some of those that segmented had still some of the follicle cells attached. In another instance 8 out of a total of 40, or 20 per cent., segmented.

These results show unmistakeably that shaking increases the percentage of self-fertilizations that take place, but whether on account of the removal of the follicle cells, or from some other change induced in the eggs is not certain.

Experiment 9. — In this case none of the shaken eggs segmented; although the crossed eggs gave 50 per cent. in one case, and 100 in another showing that the eggs and the sperm were good.

Effect of Follicle Water.

If some substance in the follicle cells excites cross-fertilization, then it seemed likely that the water in which the eggs had been violently shaken, so that the follicle cells broke apart, might act on the sperm or eggs of another individual, and cause self-fertilization. This was tried in a few cases.

Experiment 10. — In one case five per cent. of the eggs divided; in three cases there were no divisions; in another case 70 per cent. divided, but went only into the two- or four-cell stages, which seemed to show that something unusual had taken place, and that the results were not due to the follicle water. There is, in fact, a source of contamination in this experiment that may fully account for all the cases observed. In removing the eggs from the oviduct some of the sperm from the vas deferens may be accidently squeezed out and become mixed with the eggs, and remaining in the follicle water fertilize the other eggs. The shaking might injure the sperm, but those that escaped might still suffice. The peculiar segmentation in the case in which a considerable number of eggs segmented may have been due, in fact, to the injury to the sperm. Separate experiments must be undertaken to test this possibility. The negative results in these cases are probably the more significant.

Effects of Standing.

Experiment 11. — The relative age of the eggs might, it seemed to me, be a factor in the result; also by standing in sea water the substance that prevents self-fertilization might be washed out. To test these possibilities, eggs were removed and kept for eight hours in one case in sea water and then self-fertilized with their own sperm that had remained in the animal. No results followed. That the eggs are not injured by standing in sea water was shown by their power to become cross-fertilized even after 24 hours.

Effects of Bursting the Eggs.

Experiment 12. — By compressing the eggs between a cover slip and a slide they can be burst in some cases. I attempted then to self-fertilize the egg-fragments, etc. Many of the eggs that had been broken were self-fertilized, indicating that the resistance to self-fertilization is due to something in the membranes surrounding the eggs. The experiments need to be repeated on a larger scale, but even if placed beyond doubt, the explanation of what there is in the membranes that prevents self-fertilization remains still to be determined.

Extract of the Sperm.

Experiment 13. — Wishing to see if an extract of the sperm of one individual would cause the sperm of another individual to fertilize its own eggs, the following experiment was made. The sperm of three individuals was dried in the sun on a piece of glass; the residue, including the salt, was scratched off, and as much tap water was added as there had been sea water before. Sperm and eggs of another individual were then added to the solution, but no results followed.

Diluting the Water.

Experiment 14. — In order to determine whether dilution of the sea water might not bring about self-fertilization, the following experiment was made: One, two, three, four or five parts of tap water were added respectively to one hundred parts of sea water. In all of these solutions cross fertilizations took place, but in no case did self-fertilization occur.

Experiment 15. — In this case the eggs were first crossed and then put into sea water, diluted as follows: 40, 50, 60, 70 parts of tap water were added to 100 parts each of sea water. In the first and second solutions about half of the eggs segmented into several cells; in the third solution few eggs segmented, and none in the fourth, although they became polynuclear. The eggs of three individuals were put, with their own sperm, into the same solutions, but gave no results.

Summary.

1. It is not true that the sperm of a given individual will fertilize equally well the eggs of all other individuals.

2. The eggs appear to be in good condition much oftener than the sperm, but even good eggs cannot be fertilized *equally well* by good sperm of all other individuals.

3. In the case of poor sperm the discrimination shown between the eggs of different individuals is apparently more marked than in the case where the sperm is unusually good.

4. The results are not due to different amounts of sperm used in different cases, because the same, or nearly the same amount was used in each series. Other experiments showed that the amount of sperm used was many times greater than that sufficient to fertilize all the eggs present. On the other hand by using a larger amount of "poor" sperm the percentage of cases of cross-fertilization could probably be increased.

5. There is no definite relation between the number of eggs that are cross-fertilized and the normal or abnormal condition of the tadpoles. Previous experiments had also shown that perfectly normal tadpoles may be produced by self-fertilization in the few cases in which this takes place naturally or is induced.

6. If a strong extract of the ovary of an individual (A) is made, and the sperm of A (first made active in sea water) is added, and if then the eggs of B are also put in, they may not be fertilized if the solution is very strong, but if it is diluted fertilization may take place. Thus the A-sperm is not brought to rest by an A-extract, except in so far as the solution is too strong to allow any fertilization, as is shown when A-sperm is put into B-extract and B-eggs added — no fertilization taking place in the strong solution, but occurring if dilution is subsequently brought about.

7. If A-sperm is put into an extract of the ovary of B, and then A-eggs are added no fertilization occurs, showing that the extract of another individual does not excite the sperm to self-fertilization.

8. If the *eggs* of an individual (A) are placed in an extract of the ovary of B, then returned to sea-water and A-sperm added, self-fertilization does not occur. The extract of B does not effect the A-egg so that they will self-fertilize.

9. The blood and extracts of the body tissues of another individual give similar negative results.

10. Shaking the eggs, so that the follicle cells are removed, favors self-fertilization.

11. Placing the eggs and sperm of one individual in the "follicle-water" (obtained by shaking) of another individual gave distinctly increasing percentages of apparent self-fertilization, but the results are probably due to contamination.

12. Extracts of the sperm of other individuals did not, in the few cases tried, induce self-fertilization.

13. Dilution of the sea water does not facilitate self-fertilization, although cross-fertilization may still occur in water considerably diluted.

14. Eggs after standing for 24 hours in sea water are not fertilized by their own sperm.

15. Bursting the eggs, so that the membrane is ruptured or the egg is set free, allows self-fertilization to occur in a large number of cases, showing that the lack of self-fertilization of the unbroken egg is probably due to a resistance found in the surface of the egg, in the membrane, in the follicle cells or in their secretions.

Hypothetical and Speculative.

The immunity of the eggs of *Ciona* to its "own" sperm invites a comparison with cases of immunity to infectious diseases. The more so since Landsteiner and Metschnikoff have found that when, for example, spermatozoa of the ox are injected into the guinea-pig they remain active for some time, but if the injections of the ox sperm are continued at proper intervals the guinea-pig makes a spermatoxin that quickly brings to rest all later-injected spermatozoa. If something similar occurs in *Ciona* the reaction is much more delicate than any heretofore discovered, and would open a wide field for future investigation.

Without wishing at present to press this point too far it seems to me of sufficient weight to warrant calling attention to some of the implications in the case of *Ciona*.

The presence of eggs and sperm in the same individual might appear to give an opportunity for a reaction similar to that just described. If the eggs, and also the body tissue of the animal, were supposed to make a counter substance that would bring the spermatozoa to rest, we might account for the lack of self-fertilization in this way. Further, the proximity of the sperm-duct and the oviduct in *Ciona* suggests that the eggs might all the more easily be stimulated to form the substance.

There is also another possibility, viz., that a substance may be secreted by the sperm-duct that keeps the mature spermatozoa at rest, and that this substance may affect the near-lying eggs, so that they also may acquire this property. Unless, however, this influence on the eggs were of a peculiarly individual sort, it would prevent all fertilization, cross- as well as self-fertilization, which is not the case.

There remains still another possibility which may better account for the conditions. It may be that the result does not depend on a reaction between the sperm and the tissues or eggs of the same individual, but to a similarity depending on the common descent of the sperm and the eggs. Owing to their close similarity of composition, the activity of the sperm on coming in contact with its "own" egg-membranes may be decreased, so that the spermatozoön can not force its way into the egg. This point of view offers certain advantages over the others mentioned, especially when extended to some other animals. Thus in the bee, the spermatozoa are stored up in the receptaculum of the female, but the fertilization of the eggs of the female is not thereby prevented. In this case the spermatozoa and the eggs have arisen from separate individuals, and, hence, fertilization is possible, despite the fact that the sperm is stored in the body of the female that contains the eggs. In some hermaphroditic animals and plants self-fertilization occurs, but this is not a fatal objection to the hypothesis, because, although in these cases also a condition similar to that in *Ciona* may be supposed to exist, it may not be sufficiently strong to prevent the sperm from entering the eggs — the activity of the sperm being greater, or the reaction being less marked. The fact that in some of these cases self-fertilization takes place less readily than cross-fertilization is distinctly in favor of the present point of view.

The greater activity induced in the sperm of *Ciona* by ether and other exciting substances may make them sufficiently active to break through the barrier around their "own" eggs, and having once entered the egg, the stimulus caused by the nucleus or by the centrosome may cause the development to proceed.

If the spermatozoa are brought to rest by substances of some sort on the surface of the egg, or in its membranes, these may be a part of the living substance, and not set free in the sea water. Hence the failure to detect such bodies in the water or extracts under the crude condition of the experiments.

Violently shaking the eggs may remove entirely, or in part, the protecting covering, so that self-fertilization may more often take place.

If eggs are shaken in sea water, so that pieces of the follicle cells are set free, these pieces would bring to rest all of their "own" sperm that came into contact with them, but the number of spermatozoa in the water might be too great for all of them to be caught, and those that remained might suffice to fertilize the eggs of another individual if they were then added to the water. Thus we can see why in the experiments cross-fertilization was not prevented when the sperm was first added to its own follicle water.

The fact that cross-fertilization was so often incomplete in the San Diego form, and so much more frequent in the form from Woods Hole, suggests that the difference may be due to the closer relationship of the former group of individuals. The Woods Hole individuals were collected over a wider area, and other conditions at this place make it more probable that these individuals may have had a separate descent. The close resemblance, in fact, between the infertility of *Ciona* when self-fertilized, and the infertility of closely inbred forms is apparent, and the same explanation may apply to both cases.

The immunity of the eggs of *Ciona* to sperm of the same individual suggests a comparison with the cases of immunity to infectious diseases, but it is evident that the two cases can not be identical, for the antitoxin of disease is supposed to combine with the toxin of the poison, and thus inhibit its action. It would be absurd to suppose that there are bodies in the egg large enough to lock up, as it were, the spermatozoa; but on the other hand it is not impossible that substances may exist that quiet the activity of the spermatozoa, and that these substances are a part of, or the immediate product of, the protoplasm of the individual, and are not produced by a reaction between the body tissues (or the eggs) and the spermatozoa of the same individual, as in the case mentioned above when the sperm of one animal is injected into another.

A THEORY OF THE NATURE OF PROTOPLASMIC RESPIRATION AND GROWTH.

A. P. MATHEWS.

Respiration is probably the most fundamental of living processes. It is common to all forms of protoplasm; as long as it persists, protoplasm is said to live; when it ceases protoplasm dies; and it is the point of attack of the most powerful poisons such as the isocyanides. Most of the other protoplasmic functions depend directly or indirectly on respiration as, for example, the discharge of impulses from the nerve cells, the beating of the heart and so on. The protoplasmic syntheses and many of the decompositions have been ascribed since Drechsel's work and that of Hoppe-Seyler to this fundamental process.

In any general theory of respiration the following facts have to be explained:

1. Even though surrounded by oxygen all protoplasm maintains itself while alive in a reduced state and acts as an intense reducing agent. Its reducing powers are comparable to those of nascent hydrogen.

2. For all forms of protoplasm free oxygen above a certain tension is an intense poison. For some forms this tension may be no more than a very small fraction of an atmosphere; for others it is from three to four atmospheres.

3. Atmospheric oxygen has little oxidizing power; whereas protoplasm brings about oxidation of the most radical nature and is comparable in its oxidizing powers to the most intense chemical oxidizing agents.

4. Hydrogen is evolved as a gas by a great variety of bacteria and moulds.

5. The production of carbon dioxide by protoplasm stands in no direct or immediate relation to the consumption of free oxygen.

6. Many forms of protoplasm such as the anærobic bacteria are able to bring about intense oxidations in the absence of atmospheric oxygen.

The only general theory of the nature of respiration which has been carefully worked out is that of Hoppe-Seyler.[1] According to this hypothesis there occurs in the cell a fermentative decomposition of some substance analogous in all respects to the fermentative decomposition of calcium formate by many bacteria.

$$(CHO_2)_2Ca + H_2O = CaCO_3 + CO_2 + 2H_2$$

By this fermentation nascent hydrogen is set free; the nascent hydrogen keeps the protoplasm in its reduced state; it combines with one atom of the oxygen of the air to form water and sets free the other atom as nascent oxygen which thus causes the intense oxidations of protoplasm. This hypothesis was attacked by Traube, who showed in many cases that the process included the formation of hydrogen peroxide which was the real oxidizing agent. Hoppe-Seyler demonstrated, however, that some of these oxidations were more powerful than hydrogen peroxide, which is not a very strong oxidizing agent, could produce.

With the discovery of the oxidases, Hoppe-Seyler's hypothesis has fallen into the background without the oxidase hypothesis giving a better explanation in its stead. The manner of action of the oxidases is still obscure; their chemical composition is unknown, and no oxidase will cause oxidations in the absence of oxygen, whereas many forms of protoplasm can carry out such oxidations in the presence of no free oxygen at all or only of traces of free oxygen.

The studies of Armstrong, Dixon and others on the processes of slow and explosive oxidations and combustions throw, in my opinion, a remarkably clear light on protoplasmic respiration. These authors have shown that in ordinary oxidation the presence of some water is necessary to the oxidation. Phosphorus in a perfectly dry state will not ignite in dry air. Armstrong[2] concludes that the primary oxidation in all these cases is not brought about by the gaseous oxygen, but by the water. The atmospheric oxygen acts the part only of a depolarizer to take care of the nascent hydrogen formed from the water.

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[1] Hoppe-Seyler, *Physiologische Chemie*, I. Theil, Allgemeine Biologie, p. 126 ff. Berlin, 1877.

[2] Armstrong, *Chemical News*, July 15, 1904, vol. 90, p. 25; *Transactions Chemical Soc.*, vol. 63, p. 1088, 1903.

The formula for the rusting of iron on this hypothesis would be as follows :

$$\text{I.}\quad Fe + 2OH_2 = Fe(OH)_2 + 2H$$
$$\text{II.}\quad 4H + O_2 = 2H_2O \text{ or}$$
$$2H + O_2 = H_2O_2$$
$$H_2O_2 + Fe = FeO + H_2O$$

Reaction I. cannot go on unless oxygen is present to remove the nascent hydrogen. If no oxygen is present the nascent hydrogen at once reduces the iron oxide and forms metallic iron again. The atmospheric oxygen does not unite with the iron, but with the hydrogen.

The same hypothesis will explain protoplasmic respiration and at once makes clear the identity of anærobic and ærobic respiration. The following theory of respiration is founded in part on Armstrong's work.

The real respiration of all forms of protoplasm, both ærobic and anærobic, is brought about not by the oxygen of the air, but by that of the water. The hydrogen set free from the water combines with other elements of the protoplasm, thus keeping it reduced ; it also combines with the oxygen of the air if this is present to form water ; and in the absence of oxygen it may escape as free hydrogen.

The only difference between anærobic and ærobic respiration is that the anærobic protoplasm is so powerful a reducing agent that it is able to drive hydrogen out of the water, thus oxidizing itself without the aid of atmospheric oxygen to act as a depolarizer. Ærobic protoplasm being less powerfully reducing requires the presence of more or less oxygen to take care of the hydrogen. The difference between these different kinds of protoplasm is exactly the difference between metallic sodium and metallic iron. Sodium is so powerful a reducing agent that it oxidizes itself at the expense of water driving out hydrogen even in an atmosphere of hydrogen. This corresponds to anærobic protoplasm :

$$Na + HOH = NaOH + H$$

Iron is so weak a reducing agent that it requires the presence of free oxygen to take care of the hydrogen set free, before it will oxidize itself. This corresponds to ærobic respiration. Protoplasmic respiration, therefore, is in reality not the consumption of gaseous oxygen and the liberation of carbon dioxide as ordinarily stated. We now know that the production of carbon dioxide stands in no direct causal relation to the consumption of oxygen. *Respiration is in fact the dissociation of water with the liberation of hydrogen.*

The evidences of the truth of this hypothesis are many and have been collected in part by Hoppe-Seyler. Hydrogen is produced and set free as such by a great many moulds and bacteria, *i. e.*, coli communis, penicillium, butyricus, etc. In the case of facultative anærobes like the colon bacillus, the hydrogen appears only if no atmospheric oxygen is present; it is burned to water if this be present. In many cases where the hydrogen is not set free as such it escapes as marsh gas or combines with the protoplasm or some of the constituents of the culture medium, such as levulose or sulphur. Thus as Hoppe-Seyler showed, in the fermentation of calcium acetate the hydrogen unites with the methyl or methylene set free by fermentation and comes off as marsh gas.

$$(CH_3CO_2)_2Ca + H_2O = CaCO_3 + CO_2 + 2CH_4$$

In the second place hydrogen is set free in the case of some bacteria which are able to oxidize substances in the absence of oxygen. In this case the oxygen can only have come from the water. Such a case has been reported recently by Mazé.[1] In the case of a certain bacterium alcohol was oxidized to acetic acid in the absence of air, if levulose was present. The levulose was at the same time converted into mannite. The reaction was probably as follows:

$$\text{I.}\quad C_2H_5OH + H_2O = C_2H_4O_2 + 4H$$

$$\text{II.}\quad 4H + 2C_6H_{12}O_6 = \underset{\text{Mannite.}}{2C_6H_{14}O_6}$$

Reaction I. can only go on in a positive direction or from left to right if some substance is present to remove the hydrogen and thus prevent equilibrium from being established.

It will be seen that as atmospheric oxygen acts the part only of a depolarizer any other oxidizing agent, that is any other substance which unites readily with nascent hydrogen can replace the atmospheric oxygen and permit oxidation to go on in the absence of air. In the example just cited levulose acts in this way. This principle is of considerable importance in bacteriology.

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[1] Mazé, *Annales de l' Institut Pasteur*, XVII.

The hypothesis just stated necessitates, as will be seen, a total abandonment of the common interpretation of anærobic respiration as taking place at the expense of the oxygen of some food stuffs and shows at a glance the identity of the process in ærobic and anærobic forms.

What is the nature of the process going on in protoplasm by which this decomposition of water is produced?

A great number of reactions occur of just this type in organic and inorganic chemistry. One of the most striking is the oxidation of alcohol to aldehyde accompanied by the reduction of benzophenone to benzopinakon which occurs when an alcoholic solution of benzophenone is exposed to sunlight. The interpretation of this reaction as given by Nef's[1] brilliant hypothesis gives at the same time an interpretation of the living respiration which wonderfully simplifies the problem of the chemical basis of life.

According to Nef many organic reactions are brought about by a change in valence of the carbon atom from four to two. In the case just cited alcohol in the sunlight splits off water to some degree just as it does when heated and forms extremely active ethylidene particles as follows:

$$\text{I.}\quad CH_3-CH_2OH \rightarrow \underset{\text{Ethylidene.}}{CH_3-CH=} + H_2O$$

If now benzophenone is present to act as a depolarizer the active ethylidene attacks the water and oxidizes itself to aldehyde setting free nascent hydrogen which reduces the benzophenone.

$$\text{II.}\quad CH_3-CH< + OH_2 \rightarrow \underset{\text{Aldehyde.}}{CH_3CH=O} + 2H$$

$$\text{III.}\quad 2H + 2(C_6H_5)_2\underset{\downarrow}{C}-\underset{\downarrow}{O} = \underset{\text{Benzopinakone.}}{[(C_6H_5)_2CHO]_2}$$

It will be seen how exactly this process fulfills the requirements of the facts for a complete theory of respiration. Owing, therefore, to Nef's hypothesis, and without this the exact mechanism of the process would be obscure, the following picture of protoplasmic respiration may be formed :

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[1] Nef, *Liebig's Annalen*, vol. 335, 1904, p. 192.

In protoplasm there is some substance (or substances) of unknown nature which splits off water from itself just as the alcohol does in the above reaction or as ammonium hydrate does and sets free from itself active particles having the properties of ethylidene. The following three well known reactions are exactly similar to this primary process :

$$NH_4OH \rightleftharpoons H_3N < + H_2O$$
$$H_2SO_3 \rightleftharpoons O_2S < + H_2O$$
$$C_2H_5OH \rightleftharpoons CH_3 - CH < + H_2O$$

For protoplasm the reaction is as follows :

$$R - CH_2OH \rightleftharpoons R - CH < + H_2O$$

In this reaction $R—CH_2OH$ is some unknown substance.

The nature of the substances thus reacting in protoplasm is unknown. There is no evidence, however, nor is there any reason for assuming that they are complex substances. It is probably not a substance acting by means of a change in valence of nitrogen for the reason that the nitrogen substances such as ammonia do not have so great an affinity for oxygen as that required of the unknown substance. We do not get for example such a decomposition of ammonia as this :

$$NH_3 < + OH_2 = H_3NO + H_2.$$

On the other hand the substance possesses the powers ascribed by Nef to bivalent carbon. Carbon has a powerful affinity for oxygen. It may well be, therefore, that the substance is a carbon compound, presumably of simple nature and derived directly or indirectly from the foods so that new raw material for the reaction is constantly supplied. If this is the case Nef's view that bivalent carbon is at the bottom of the vital reaction would be justified.[1]

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[1] Probably all reducing agents act in some measure in this way. For example in egg white a substance is present which forms sulphuretted hydrogen in the presence of sulphur. This substance is of unknown nature. The reaction probably is a follows :

$$X - CH < + OH_2 = XCHO + 2H$$
$$2H + S = H_2S$$

Whatever their composition may be the active particles attack the water of the protoplasm, oxidize themselves to aldehydes and set free nascent hydrogen.

$$R - CH < + OH_2 = R - CHO + 2H.$$

This explains the presence of aldehydes in protoplasm; the production of hydrogen by many or all forms of protoplasm as assumed by Hoppe-Seyler and the keeping of the cell protoplasm in a reduced state. It is perfectly clear that surrounded by oxygen as most protoplasm is it could not possibly continue in its reduced condition if some strong reducing agent such as hydrogen was not constantly produced. If too much free oxygen is present the aldehydes will be converted into acids, the reaction of the protoplasm changed and the life of the cell destroyed. Oxygen in other words above a certain pressure is poisonous for protoplasm.

In those forms of protoplasm called anærobic the active particles are of such a character and so powerful that in the absence of oxygen they can like sodium, oxidize themselves and set free hydrogen; or in the presence of certain foods which will combine with hydrogen such as levulose, they are able to oxidize themselves. In those forms of protoplasm called ærobic the active particles are not so powerful and require the aid of atmospheric oxygen to combine with the hydrogen before they can decompose water. While the active particles are formed spontaneously, the conditions in protoplasm may be such as to accelerate their formation (ferment action, see Nef).

A few examples will perhaps make clear the possible relations of this fundamental reaction to the synthetic properties of the protoplasm. The fundamental nature of most of these syntheses is simple and consists in the elimination of water between two simple molecules to form a complex molecule, as for example, in the condensation of the amino acids to form albumin; of the monosaccharids to form disaccharids and polysaccharids; of the amino acids to form pyrimidin; of the alcohols and fatty acids to form fats; of ammonia and oxyacids to form amino acids.

Two explanations of this process may be given. That of Drechsel is the first. According to this theory the elements to form water are eliminated by a successive oxidation and reduction of the substances. A type of this reaction is the conversion of ammonium carbamate into urea.

$$NH_4OCONH_2 - O = NH_2OCONH_2 + H_2O$$
$$NH_2OCONH_2 + 2H = \underset{\text{Urea.}}{NH_2CONH_2} + H_2O.$$

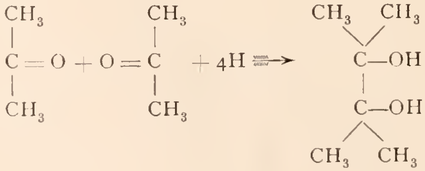
Upon the basis of this hypothesis, Drechsel succeeded outside the cell by rapidly alternating reductions and oxidations produced by induction shocks in obtaining urea from albumin ; in synthesizing hippuric acid from benzoic acid and glycocoll, and in synthesizing ethereal sulphates. These syntheses may thus very readily be the secondary result of that fundamental respiratory process characterized as it is by a simultaneous reduction and oxidation.

The syntheses may also be due as shown by Nef for many reactions to the condensation of active particles, that is particles formed by the dissociation of organic substances and containing open valencies. A typical synthesis of this sort would be the formation of olefine gas from methyl alcohol, or the condensation of the isocyanides.

$$\text{I.}\quad 2H \cdot NC \rightarrow HNC< + HNC< \rightarrow H_2N_2C_2$$
$$\text{II.}\quad 2CH_3OH \rightarrow 2CH_2< + 2H_2O \rightarrow \underset{\text{Olefine.}}{C_2H_4} + 2H_2O$$

If in protoplasm conditions are such as to cause a good many active particles to be produced, syntheses by condensations of all kinds must spontaneously and of necessity occur. In this way it is seen that both growth and respiration are due to the spontaneous formation of active particles from the foods.

In the third place, mere reduction may cause many syntheses. A typical example of this sort is the condensation of acetone by nascent hydrogen.

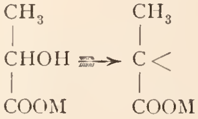


Acetone. Pinacone.

In this case a forked chain is produced, in which the carbon atoms are attached directly.

Moreover, from the aldehydes produced in the way indicated, all manner of compounds will originate spontaneously, as Loew has particularly pointed out. Thus from formaldehyde, formose and the sugars spontaneously originate in faintly alkaline solutions; glycerine aldehyde goes over of itself into a hexose sugar; acetic aldehyde in the presence of ammonium cyanide forms propionic amido-nitril, which readily passes into amido propionic acid or alanin, one of the most common constituents of the albumins. The albumins themselves are nothing else, as Kossel and Fischer have shown, than condensed amino acids. This condensation, there is every reason to believe, can be brought about by cell extracts, since from the kidney a substance has been isolated which brings about identically the same kind of a condensation, namely, that of benzoic acid and glycocoll, to form hippuric acid. Finally, by spontaneous union of the aldehydes with the cyanides found in so many cells, carbon chains are built up readily outside the cell and presumably in the cell also, since nitrils are not uncommon constituents of protoplasm.

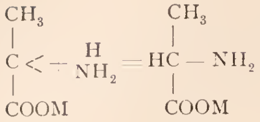
Many other examples might be given here, but these will, I think, indicate how completely the formation of substances found in protoplasm can be accounted for by means of this fundamental reaction which is going on. These syntheses and decompositions must take place as a matter of course if the reaction is of the nature sketched. I do not mean to imply that the amino acids, for example originate altogether in the manner indicated. They may also be formed by the union of oxyacids dissociated in Nef's sense with NH_3, without the intermediate formation of aldehydes or ketones. Nef has shown that the lactates for example dissociate as follows:



$$\begin{array}{l} CH_3 \\ | \\ CHOH \\ | \\ COOM \end{array} \longrightarrow \begin{array}{l} CH_3 \\ | \\ C< \\ | \\ COOM \end{array}$$

Lactate.

If now NH_3 is present, union takes place directly with the bivalent carbon atom.



$$\underset{\underset{COOM}{|}}{\overset{\overset{CH_3}{|}}{C}} < + \begin{matrix}H\\NH_2\end{matrix} = \underset{\underset{COOM}{|}}{\overset{\overset{CH_3}{|}}{HC}} - NH_2$$

Alanin.

In any case it will be apparent that the synthetic powers of the cell depend on the same process as the respiratory powers.

While the interpretation of the exact mechanism by which the respiratory substance or substances (for there may be many such substances in one cell) dissociate water depends, as will be seen, upon Nef's hypothesis and is due to this hypothesis, the general hypothesis that such dissociation of water is taking place and that this is the basis of respiration, is independent of Nef's hypothesis. If, however, that hypothesis be accepted, and of its truth he has already produced so many proofs as to entitle it to provisional acceptance, we can go farther and ascribe this most fundamental of living reactions and many protoplasmic syntheses to methylene dissociation, that is, to bivalent carbon particles in protoplasm, as Nef himself has pointed out. Protoplasmic respiration must in any case be defined in the light of known facts somewhat differently from the ordinary statement.

Respiration is that process going on in protoplasm by which water is decomposed into oxygen and hydrogen. The oxygen combines with the substances of the protoplasm thus oxidizing them ; the hydrogen is either set free in the gaseous form, or it is united with atmospheric oxygen to form water ; or it combines with other substances in the protoplasm.

The reaction may be written as follows :

For anærobic protoplasm :

I. $R-CH_2OH \rightarrow R-CH< + H_2O$

II. $R-CH< + OH_2 \rightarrow R-CH=O + H_2$[1]

For ærobic protoplasm :

I. $R'-CH_2OH \rightarrow R'-CH< + H_2O$

II. $R'-CH< + OH_2 + O \rightarrow R'CH=O + H_2O$, or

III. $R'-CH< + OH_2 + O_2 \rightarrow R'CH=O + H_2O_2$

IV. $R'-CH< + H_2O_2 \rightarrow R'-CH=O + H_2O$

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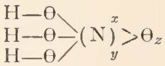
[1] A great number of reactions of this type are given in Nef's paper already referred to. See also Nef, *Journal of the American Chemical Society*, Vol. XXVI., 1904, p. 1549.

The views expressed in this paper may be harmonized with those in a previous paper in which the oxidations and reductions going on in protoplasm were regarded as electrical,[1] if the following explanation of the dissociation of NH_4OH into NH_3 and H_2O be adopted. At present no clear connection between ionic dissociation and such dissociations as that of ammonia has been established. The relation of the two processes may be the following, if Thompson's electron hypothesis be adopted.

In sodium chloride the sodium and chlorine are held together by the affinity each has for a negative electron. $Na—\theta—Cl$. On going into aqueous solution the atoms separate and chlorine having the greater affinity for the electron (+ 1.965 volts to — 2.54 volts for the sodium in normal solution) takes it away from the sodium. The sodium being thus left with an unsaturated affinity for a negative charge becomes a positive ion ; the chlorine with a free negative charge becomes a negative ion. The free charges being on separate atoms and free to move the solution conducts the current. In the albumin molecule of amphoter reaction hydrogen ions are dissociated at one place ; hydroxyl at another. A free positive charge and a free negative charge reside on the same molecule though on different atoms. The albumin molecule may, therefore, be unable to take part in the conduction of the current although ionized.

$$\mathrm{H}\overset{+}{—}\quad \theta—\text{albumin}\overset{+}{—}\quad \theta—\mathrm{OH}$$

In the nitrogen atom as it exists in ammonia the opposite charges are on the same atom. The condition may be represented as follows where nitrogen is trivalent.



If N is the nitrogen atom it has three valencies satisfied by hydrogen. At x and y, two points on its surface, there is a positive charge not entirely compensated by the atom itself. This charge is compensated by the electron z. This electron may be regarded as holding together the two parts of the nitrogen atom at x and y, just as the electron held together the separate atoms of sodium and chlorine. In this case, I assume, the condition is that of Nef's polarized valencies. Particles in this condition he assumes to be inert so far as valencies *x* and *y* are concerned. Nef assumes that these polarized valencies open up and they open up more readily under some conditions than under others. For example, the polarized valencies of the carbon atom open up when the carbon is joined to nitrogen as in the isocyanides far more easily than they do when the carbon is joined to oxygen, as in carbon monoxide. The opening up of the valence is, I think, identical in character with the dissociation of a salt into its ions. The opening of these valencies constitutes a dissociation of valencies. It makes, of course, no difference theoretically whether those valencies are on the same atom or on different atoms. At any rate NH_3 goes over into:

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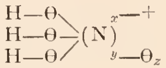
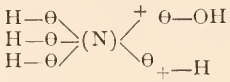
[1] Mathews, *Amer. Journal of Physiology*, 1904, X., p. 290.



These are Nef's active particles. It is only when in this condition of dissociation that these valencies will combine. One of these valencies (*x*) is positive; the other (*y*) is negative. Such particles, although they are really in an ionic form, cannot conduct the current because they are positive and negative at the same time.

These particles combine as follows:



Exactly the same reasoning applies to all atoms, including bivalent carbon. It will be seen, therefore, that this explanation which is but a trifling addition to the fundamental idea of Nef of polarized valencies, would bring the respiratory reactions into the domain of electrical reactions. No doubt this explanation has occurred to others, and I give it only as a convenient picture. It bridges so well the gap between Nef's views of organic reactions and Ostwald's hypothesis that inorganic reactions are ionic that it may be useful.

It will be noticed also that upon this view residual valencies may be pictured as polarized valencies and in each case both positive and negative valencies are opened simultaneously. The number of residual valencies may be considerable since upon Thompson's hypothesis of the electronic constitution of matter the movement outward of any electron owing to the speed of rotation of the atom or a disturbance of its electrical equilibrium would open up such valencies. In fact, the whole atom would be made up of polarized valencies if we look at it in this way.

Summary and Conclusion.

The foregoing hypothesis has a close bearing on the spontaneous origin of living matter. As a result of chemical and histological work it is now clear that living matter is a mixture of various substances and is not a chemical compound ; there is no living compound. While this conclusion may not be admitted by all, it is, in my opinion, clearly established. There is no longer any doubt that the different phenomena of life are due to different constituents in this mixture. The histological, physiological, pharmacological and chemical evidence is unanimous on this point. The problem of the origin of life no longer appears, therefore, under its former guise, of how the foods are transformed into a living molecule or compound, but in its place we have instead, how are the foods transformed into the mixture of substances which is called living matter.

The question which we have to answer is this : is the transformation of the foods into living matter produced by the foods ; or is it produced by the living matter ? At first glance everyone would say that the transformation was caused by the living matter. This is the answer everyone has given hitherto and its truth appears to be self-evident. If we mix the foods by themselves they do not produce living matter. This answer has made it difficult to see how living matter originated on the earth. Preëxisting living matter was always needed to get living matter.

In the light of the foregoing discussion and the study of the transformations of the foods in protoplasm it appears highly probable that this answer is erroneous. Living matter does not cause the transformation of foods into living matter as we imagine ; instead the foods to-day spontaneously change themselves into living matter just as they did at the beginning. The change goes on very rapidly in living matter, but very slowly outside.

The cause of the formation of living matter is to be sought in the foods and not in the living matter.

As this conclusion has not so far as I know been hitherto perceived, the reasons for it may be briefly presented. They are in brief two: The examination of protoplasm has shown that it contains a great number of catalyzing agents or ferments. So many of these have been found in all cells and so clearly do protoplasmic reactions partake of this nature that the opinion is widely accepted that the chemical transformation of the foods in protoplasm into the substances constituting protoplasm is brought about by ferments. The work which has been done by the physical chemists and others upon ferments shows in the clearest manner that ferments are substances which do not cause reactions, they only accelerate reactions which will go on anyway in their absence, but which go on very slowly. Ferments therefore are accelerators of spontaneous reactions. Zymase does not cause the decomposition of the sugar molecule, it only accelerates its decomposition. The necessary result of this fundamental conception of the nature of ferment actions is this: the nature of the chemical transformations which the foods undergo in protoplasm is not altered or determined by the protoplasm. The nature of the transformation is determined by the foods; protoplasm by means of the ferments it contains only influences the *rate* of the transformation. This means as already pointed out that the foods must spontaneously transform themselves into the mixture called protoplasm, if given time enough. In protoplasm this transformation goes on very rapidly because some of the products of the reaction act as catalyzers to hasten the rate of this or that phase of the reaction.

In the second place a careful study of the transformations of the foods within and outside of protoplasm has failed to show a single instance in which the character of the transformation is different in two cases. This result is now so well understood in physiological chemistry that if we wish to discover what substances are formed out of any food or other substance during its passage through protoplasm, we subject the substance to decompositions, hydrolytic, oxidative, or reducing, outside the body, determine the substances formed and then look for these substances in the organism knowing that they will be formed there also.

In the third place a mass of evidence shows that substances outside the body simply dissolved in water, break up at a very slow rate, but into the same compounds in many instances as appear at a rapid rate in protoplasm.

These facts may be summarized in the general statement: Living matter does not determine the *character* of the transformation of the foods; it only determines the *rate* of transformation.

This conclusion at once makes clear that living matter is to-day originating spontaneously from the foods just as it always has. The transformation goes on now very rapidly owing to catalyzers in protoplasm, whereas originally the transformation probably went on slowly until some of the products of the reaction were produced which acted as catalyzers for this or that phase. What we call living matter is of importance in the process only because it contains from the outset these end-product catalyzers, formed from the previous reaction. The whole process is, I conceive, as follows:

The carbon constitutents of the foods spontaneously decompose. By this decomposition particles in a nascent state are formed. Upon Nef's hypothesis these would be particles with bivalent carbon. Whatever their nature these nascent particles either act on the water, oxidizing themselves to aldehydes or ketones and setting free hydrogen and in this manner causing protoplasmic respiration; or they combine with each other to form the various constituents of protoplasm and thus cause growth. What they combine with depends on what substance is near when they become nascent. If it is ammonia amids, amino-acids and other nitrogen compounds are formed; if another carbon compound, the carbon chains are built up and the complex substances which make up protoplasm. The whole process is due to a spontaneous dissociation of the food molecules and is not due to any vital energy. Some of the end-products of this spontaneous rearrangement act as ferments, that is, as accelerators of some phase of the reaction, or they may act as negative catalyzers delaying some phase. Examples of such processes are well known in chemical reactions. In exactly what manner they accelerate or delay the reaction is not certain.

The bearings of this conclusion upon many problems of biology, and particularly upon the problem of differentiation in development is obvious. The substances produced from the foods depend on the foods and not on the protoplasm. The so-called organ-forming substances of the egg described by Whitman, Lillie, Conklin and others, determine necessarily the character of the protoplasm formed from them. There is but one other factor to be considered, *i. e.*, the presence of catalyzers which may accelerate different phases of a reaction. By this means a food substance, undergoing in many different cells the same course of transformation, will give rise in each to different proportions of substances, depending upon what stage of its transformation is accelerated. Thus, for example, a sugar undergoing one transformation into alcohol, lactic acid and carbon dioxide, or other substances may very readily give rise almost exclusively to lactic acid, if the proper phase is accelerated.

In this way, it is possible to see how the same foods in different protoplasms will form substances which are present in the different cells in widely different proportions. A specific instance will make this clear. Amino-acids spontaneously split off the amid group and form oxyacids. This occurs at a very slow rate and is a reversible change. In the liver of mammals there is a catalyzer which greatly accelerates this transformation. The result is that in this organ quantities of ammonia are produced and a non-nitrogenous residue. In cells lacking this ferment, this reaction goes on so slowly that there is no opportunity for the accumulation and farther decomposition and recombination of the products thus set free. Numerous other examples will occur to all physiological chemists.

In conclusion, I wish to point out that this conception is in many important particulars only an application to some of the problems of physiological chemistry of Nef's theory of the nature of organic chemical reactions. As will be seen from this paper, I believe that his hypothesis of the spontaneous decomposition of organic molecules with the formation of extremely reactive dissociation products throws a new light on the chemical transformations in protoplasm, and that many of those reactions become clear at once if it be assumed that the reactive particles have the properties of bivalent carbon. The importance of this theory for physiological chemistry is not yet sufficiently recognized.

ON THE NUCLEOLI IN THE SOMATIC AND GERM CELLS OF PEDICELLINA AMERICANA.

LOUIS I. DUBLIN.

In the course of a work on the germ cells of *Pedicellina americana*, just published, (Dublin, '05), my attention was called among other matters to the study of the character of the nucleolus, not only in the various stages in the differentiation of the germ cells, but in the somatic cells as well. Thus, in the resting condition of the cell, the nucleoli are nearly everywhere in pairs and are situated at opposite points near the nuclear membrane. This arrangement is so constant, as are also the later processes through which these bodies pass, that the possibility was immediately suggested that *Pedicellina* might be one more instance among the many cited by Häcker, '02, where there is a distinct outward expression of the internal independence of the paternal and maternal elements, not only in the nuclei of the early somatic cells, as is most clearly observed in several forms, but also in the early germ-cells. It was therefore determined to put this whole question to a test by an examination of the cells throughout the whole life cycle, *viz.*, to observe whether the condition of the chromatin was actually in accordance with the conditions so strongly suggested by the appearance and behavior of the nucleoli.

For this purpose *Pedicellina* presents very favorable conditions. In the first place, owing to the comparatively small size of the polyps, the various tissues including the ovaries and testes, can be brought into the same microscopic view, thus affording very close comparison of these structures throughout. In the second place, and more important, owing to the nature of the budding process and the internal development of the embryo, the cells of all the stages of the life history are easily accessible for study with the exception of a short and, for our purpose, rather unimportant period during metamorphosis when the free swimming larva leaves the brood-pouch and becomes attached. Such a study of the entire life history is, finally, all the more important in the light of the discussion on the nature of the nucleoli and of the relation which the latter bear to the chromatic substance. I shall then, first, review the character of the nucleoli throughout the life history and, second, limit myself to the nucleolus of the growing oöcyte.

I.

The youngest polypides at my disposal were those which had but lately passed through their metamorphosis (Fig. 1). These in many cases do not as yet show any traces of the budding stolon and have certainly no genetic connection with the colonies among which they are found. There is still no apparent distinction of sex and the internal structures are at the beginning of their development. Of the digestive system, only the middle portion has definitely formed. The atrium or vestibule is represented by a small space which has as yet no connection with the outside. Surrounding the gut and vestibule and filling the space between these parts and the body wall are masses of embryonic cells which later give rise to the ovary or testis to the nervous and excretory systems, the œsophagus and rectum, the tentacles and the few muscle bands. These embryonic cells are all of one main type and cannot as yet be distinguished very positively from the primitive germ-cells which lie in their midst. One character, however, stands out strikingly. The comparatively large nuclei, in the great majority of cases, contain each two nucleoli. These two bodies are located symmetrically with respect to the halves of the nucleus. In the remaining cases there is but one nucleolus and this one is almost invariably of larger size than either of the two and is located at or very near the center of the nucleus.

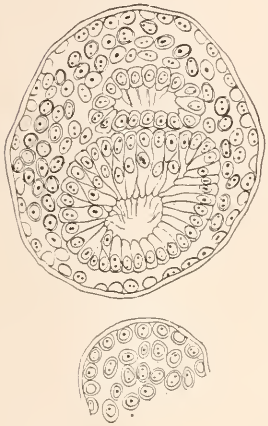


FIG. 1.

In a somewhat later stage, the stalk is longer and the internal organs begin to show something of their permanent form. The primitive germ-cells, which can now be distinguished very clearly, have taken their final place between the "liver-cells" and the floor of the atrium and there multiply very rapidly. This increase is much more pronounced in the male than in the female and gives the first and principal basis for the distinction now present between the sexes. The bi-partite testis, with its spermatogonia, has grown considerably and the latter are mostly in the resting condition. The nuclei are large, being surrounded by a very thin layer of cytoplasm. Within the nucleus, the chromatin, in the form of a reticulum, stains very lightly; indeed, in well extracted iron-hæmatoxylin preparations shows scarcely at all. But what is more important is the almost invariable presence of the one or the two nucleoli. These stain uniformly and intensely black with hæmotoxylin and retain their color long after that of the other cell elements has been extracted. When in pairs, they are placed at opposite points of the nucleus; when single, the nucleolus is found more nearly in the middle. The cells of the other tissues have preserved the same condition of the nuclei as was above described for the younger polyp.

In older testes, many more cells are present (Fig. 2), and these are proved, by the unreduced number of the chromosomes, to be also spermatogonia. Among these are cells of many sizes both at rest and in mitosis from which it is certain that there are several generations of spermatogonia. In tracing the development of the spermatogonium from the telophase of the preceding division, attention is attracted by the appearance of the two nucleoli. At the beginning of the reconstruction there is as yet no trace of them. This stage is soon followed by one where the nuclear membrane has reappeared, the chromatin being almost entirely unaffected by hæmatoxylin. At two opposite points of the nucleus there now appear the two nucleoli as yet very small but

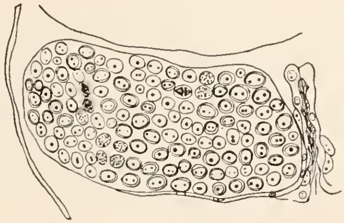


FIG. 2.

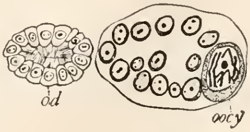


FIG. 3.

deeply staining, very much like the granule of a centrosome. It is important to observe, that the number is at this point always two, the one larger nucleolus occurring only in the later stages of spermatogonial development. The chromatin soon increases in staining power, the nuclear membrane stands out more clearly and the two nucleoli increase in size until they become very conspicious. In the Auerbach preparations, the green chromatin reticulum near the nuclear membrane stands out rather clearly from the two red plastin nucleoli. These, at this point, approach the center of the nucleus and finally fuse into one larger nucleolus. All the stages in the approach of the two are to be found in one microscopic field in almost any testis. The appearance of these bodies as they come into closer relation, indicates that there is a flowing of their liquid substance toward a common center. In this way is produced a fine strand, deeply staining, which connects them before they have fused. We can now more readily understand the prevalence of the single nucleoli in resting spermatogonia. They represent the two, which, originally distinct, have now fused. It must here be added, however, that there is some doubt whether the two always fuse into one. There is evidence for the possibility that, in some few cases at least, the two remain distinct until the formation of the next spindle figure when they disintegrate.

The chromatin by this time stains much more intensely and takes the form of definite chromosomes. These may now move from the periphery and come into closer relation with the one or the two nucleoli. This connection is, however, always a secondary one, and in no case represents the origin of chromosomes from chromatin nucleoli. There can be little doubt of this conclusion, for on staining with Auerbach's fluid the chromatin stains intensely green while the nucleoli are always red. The same decisive results were obtained with the use of the Borel stain.

The oögonia at first resemble the spermatogonia very closely (Fig. 3) but can be distinguished, even at an early period, by their smaller number. The chromatin goes through exactly the same transformation in the several generations as do also the two nucleoli. In their early appearance at or near opposite points on the newly formed nuclear membrane, in their subsequent growth and movement toward the center, and finally, in their fusion, the resemblance is quite complete. We may therefore infer that whatever be the significance of the regularity of the occurrence of these bodies in the males must also hold in the case of the females.

This striking condition of the nucleoli of the primary germ-cells (Ur-genital Zellen) and of the several generations of spermatogonia and oögonia can be no mere chance occurrence. In the testes of older individuals, where some of the spermatocytes have already arisen, nearly every resting spermatogonial cell shows the primarily double or the secondarily fused condition of the nucleoli. This period closes, however, with the spermatogonic cycle since the spermatocytic divisions follow without an intervening resting stage. In the ovary, where the last oögonial division is followed by a long period of growth, the young oöcytes also show the same condition of the nucleoli that has been described for the oögonia. These either persist separately or fuse, but, unlike the nucleoli of the earlier germ-cells, become the composite structures generally found in the nearly matured egg. To this, however, we shall turn below.

As was pointed out in the discussion of the young polypides, where the internal tissues are not as yet completely differentiated, the primary germ-cells are not to be distinguished either in size or form from the other embryonic cells. These latter also show the same double nucleoli and act in other respects as do the germ-cells. From this we might expect to find similar conditions in the several tissues of the body to which the embryonic cells give rise, and such is, in fact, the case. In polyps, old or young, in males or in females, every organ shows the same con-

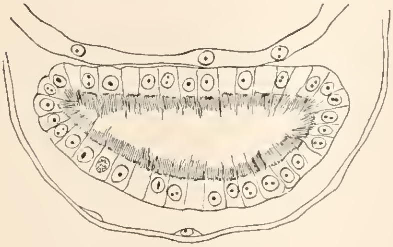


FIG. 4.

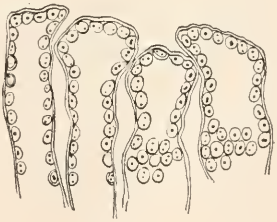


FIG. 5.

stitution of the nuclei. In the highly-modified "liver-cells," in the epithelium of the gut (Fig. 4), in the cells of the atrium and tentacles (Fig. 5), the primitive conditions are alike preserved.

From the universality of the occurrence of this phenomenon, it is difficult to escape the conviction that we are here concerned with conditions of considerable importance in the ultimate constitution of the nucleus. We must now inquire what light the fertilization and the early cleavage stages will throw on the problem ; for it is here that the constitution of the nucleus ought most readily to be made out. In the first place, the two pronuclei do not completely fuse in the act of fertilization. In this regard, *Pedicellina* recalls the condition found by many observers in other forms. Indeed, the individual chromosomes are often formed in both pronuclei before their apposition has occurred and in no case is there much, if any possibility of their fusion during fertilization. The nuclei are extremely large and apparently correlated with this is a fact that each contains many nucleoli ; the number varying from two to as many as seven or more (Fig. 6). In the 2- (Fig. 7), 4-, and 8-cell stages, the same conditions with reference to the nucleoli exist. As the cleavages go on a little further the conditions become more in accordance with what was observed in the latter somatic stages. The nuclei become successively reduced in number and it is no uncommon occurrence to find in a 16-cell stage as many as half the cells with two nucleoli (Fig. 8). These two may fuse into one, as is the case in the oögonia and spermatogonia already described. In the 32–64-cell stage, the mono- or bi-nucleolate condition is already the all-prevailing one, all the stages in the approximation and fusion of the two into one being present (Fig. 9). From this point forward, I have never found, in any of the many em-

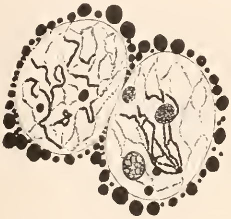


Fig. 6.

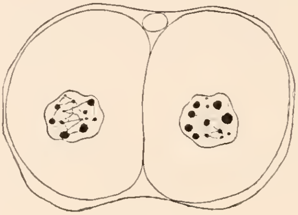


Fig. 7.

bryos examined, more than two nucleoli and these go through all the typical transformations already described. The same conditions are obtained in all the resting cells of the later embryos up to and including the free-swimming larva (Fig. 10). After the fixation of the latter, and the ensuing metamorphosis, the young polypide, before stolon formation, shows the same interesting conditions. We have thus traced the constitution of the nuclei from the early polyp through the maturer ones, into the formation of the germ-cells and finally, in the new generation, have passed through the cleavage stages up to the formation of the larva and have completed the cycle with the fixed polyp once more.

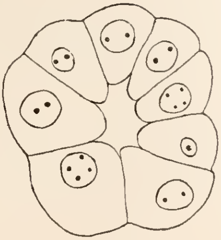


Fig. 8.

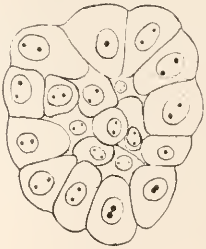


Fig. 9.

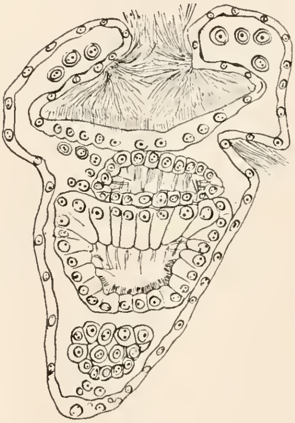


Fig. 10.

From this evidence, coupled with that of Rückert, '95, Häcker, '95–'02, Zoja, '95, Herla, '93, Conklin, '01–'02, and most recently of Moenkhaus, '04, one might at first thought conclude that in *Pedicellina* as in the Copepods, etc., the nucleoli actually express the internal relations of the parental chromatins of the nucleus and in their duality represent the persistent segregation and autonomy of the paternal and maternal chromosomes. This conclusion, however attractive, has on close examination of the evidence not only in *Pedicellina*, but in the other forms as well, a source of serious difficulty. From the recent work of Moenkhaus, '04, above mentioned, it is clear that while the two nucleoli may be very generally present in the late cleavage stages of the hybrid teleost embryo, the parental chromosomes are positively not separated into two groups. Thus during and after the third cleavage of the egg, the long and the short chromosomes of the two parents of the hybrid mingle together, the spindles never again showing any division into right and left sides with the corresponding separation of the chromosomes, according to the work of Häcker, so marked in the early divisions of the copepod egg. The double nucleolate condition, cannot, therefore, be taken as an indication of the autonomy of the paternal and maternal contributions.

In *Pedicellina*, moreover, the fertilization and early cleavage stages, where the autonomy of the parental chromatins would be most marked if at all present, is not at all in evidence. In spite of the bi-nucleolate condition of the later life history, it is clear from the early stages that the chromosomes of the egg and the sperm have, as in the Fundulus-Menidia hybrid of Moenkhaus, entirely mingled, perhaps in the first cleavage spindle. Thus, also in *Ascaris*, according to Zoja, '95, where the chromosomes of the egg and sperm have mingled as early as the 12-cell stage. In the Copepods themselves, it is not clear that the physiological distinction in the parental sides of the spindles persists after the early cleavages, and thus, the only important evidence for persistent gonomery is, in these types, the constant appearance of the two nucleoli, and this, it is clear, involves an assumption which, in view of the latest work, is full of difficulties. Of still less weight is the occurrence of bilobed nuclei in the late stages, these probably representing little more than an intermediate stage in the fusion of the several vesicles into one resting nucleus. The appearance of the double spirem in the early germ-cells while of greater value is, from the rarity of its occurrence and from the want of proof that the halves are parentally different, also indecisive.

It is therefore much more probable that the parental chromosomes actually mingle among themselves at an early period of development, and that these so-called outward expressions of internal independence are either accidental and unconnected with the chromatin phenomena or are expressive of other conditions within the nucleus. By this it is not meant that the chromosomes lose their individuality or fuse into one indiscriminate mass. On the contrary, in most forms examined on this matter and in *Pedicellina* particularly, the persistence of this individuality is most marked although it is impossible to distinguish between the paternal and maternal chromosomes now mingled among themselves.

This conclusion is not only more in accordance with the facts but can alone directly explain the possibility of a parental synapsis, *i. e.*, the union of the homologous paternal and maternal chromosomes into pairs, thus reducing the number to one half at the close of the oögonic and spermatogonic cycles in the early germ-cells (Montgomery and Sutton). Were the homologous chromosomes segregated at this period also, this union would be impossible; mingled together, however, as Moenkhaus has actually shown in the teleost and as is very probable in other forms, the union would be readily effected.

What then is the significance of the two nucleoli so constant in the life history of *Pedicellina?* In the present state of our knowledge, when such uncertainty as to the nature and function of the nucleolus still persists, no definite answer can be given. In *Pedicellina* it is clear that they are not chromatin bodies and come only incidentally into connection with the chromosomes. They are plastin bodies in some way, very probably, connected with the waste products of division, as Häcker's theory maintains. Yet, whatever may be the significance of the nucleoli as organs of the cell, they do in some way reflect or represent the activities of the nuclear areas in which they arise. When, therefore, we find in the cells of the cleavage and later stages two nucleoli removed to opposite portions of the nucleus we may perhaps be permitted to infer that these are the outward expressions of the activities of these two portions which together compose the nucleus, and if an interpretation of parental nuclear autonomy be at all justified by the facts, and this is very questionable, I should suggest that it is much more probable that this autonomy applies to the paternal and maternal sap or ground substance in which the chromosomes are distributed.

In this connection, there is one more consideration to which attention must be drawn. In the spermatid of *Pedicellina*, the chromatin appears in two masses; one localized at the anterior, the other at the posterior end of the now elongated sperm head, there being a non-staining middle space between (Fig. 11). On such a condition as this one might extend to this post-maturation stage the same interpretation that was criticized above. Indeed Häcker would so conclude. In his '02 work, referred to above, this author is quite ready to interpret the presence of two nucleoli in the matured sperm head as an indication of the persistent autonomy of the parental chromatin halves, even until this period. The only uncertainty, as he admits, in the Copepods under consideration (*Diaptomus* and *Heterocope*) being the presence of three or four nucleoli; nor are these placed at two opposite points of the nucleus as his hypothesis of gonomery demands.

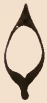


FIG. 11.

In the matured egg, however, he finds absolutely no difficulty in accepting this conclusion. Speaking of the chromosomes at the beginning of the second maturation division, he states, p. 343: "Die 12 neuformirten bivalenten Elemente . . . werden durch den Reduktionsakt auf der zweiten Richtungskörper und Eizelle verteilt und letzterer enthält demnach 6 Mischlinge, welche sich je aus einer väterlichen und mütterlichen, oder, da die reife Eizelle bereits eine neue Generation repräsentiert, besser gesagt, aus einer groszväterlichen und groszmütterlichen Hälfte zusammensetzen." To understand fully what this post-maturation gonomery of Häcker involves, it is necessary to review in more detail the processes by which it is attained. In a late prophase of the first maturation mitosis, the twenty-four chromosomes (the somatic number) appear in two rows of six pairs each; the paired bivalent chromosomes having arisen through the union end to end of single chromosomes of the same parental side. Thus, the twelve paternal chromosomes, *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, *j*, *k*, *l*, are arranged *ab*, *cd*, *ef*, *gh*, *ij*, *kl*, while correspondingly those of the maternal side as *no*, *pq*, *rs*, *tu*, *vw*, *xy*. Each of the pairs then splits longitudinally forming twelve tetrads, six completely paternal and six completely maternal. The first maturation which is equational reduces the tetrads to dyads leaving in the egg twelve bivalent chromosomes, six from each parental side. Now ensues a second synapsis between pairs, this time of

opposite parental sides, thus giving rise to six tetrads, viz; : $\begin{array}{c|c} a & b \\ \hline n & o \end{array}$, $\begin{array}{c|c} c & d \\ \hline p & q \end{array}$, $\begin{array}{c|c} c & f \\ \hline r & s \end{array}$, $\begin{array}{c|c} g & h \\ \hline t & u \end{array}$, $\begin{array}{c|c} i & j \\ \hline v & w \end{array}$, $\begin{array}{c|c} k & l \\ \hline x & y \end{array}$, and through a subsequent rotation these become $\begin{array}{c|c} a & n \\ \hline b & o \end{array}$, $\begin{array}{c|c} c & p \\ \hline d & q \end{array}$, $\begin{array}{c|c} e & r \\ \hline f & s \end{array}$, $\begin{array}{c|c} g & t \\ \hline h & u \end{array}$, $\begin{array}{c|c} i & v \\ \hline j & w \end{array}$, $\begin{array}{c|c} k & x \\ \hline l & y \end{array}$. The second or reducing division distributes these in such a manner that the egg and the second polar body each receives twelve chromosomes (six bivalents) of which six single elements are paternal and six maternal ; thus, *an*, *cp*, *er*, *gt*, *iv*, *kx*, go to the now matured egg while the rest go to the second polar body.

There is, however, but little probability for such an interpretation as it entirely ignores the significance of the primary synapsis which from the figures of Rückert, '04, and Häcker, '95, himself, undoubtedly takes place at the beginning of oöcytic growth. This undoubtedly results in the reduction of the chromosomes during this period. From the highly probably results of Montgomery and Sutten (which have received strong corroboration from all sides) the twelve bivalents of the first maturation prophase would represent the union of homologous paternal and maternal chromosomes and not, as Häcker assumes, of the same parental side, *i. e.*, paternal with paternal, maternal with maternal On the first of these hypotheses the two ensuing maturation divisions would produce conditions quite analogous to that observed by the great majority of workers on the germ-cells of both animals and plants. But what is far more convincing is the fact, that on the interpretation of Häcker, there would result, in the great majority of cases, an egg with such a chromatic constitution that the potentialities of one group of characters would often be doubled, while those of another group would be entirely lacking. This would not only make Mendelian results impossible but, as Boveri has shown in his work on "Multipolar-mitoses" might lead to the production of defective larvæ.

We must therefore conclude that the segregation of the chromatin in the spermatid of *Pedicellina* is without particular significance and that the gonomery or autonomy of the parental chromatic contributions continues at most only up to the beginning of the growth period of the germs-cells, the mingling having occurred, in most cases, long before this. At this point, in the processes of synapsis the several chromosomes of the paternal side very probably individually unite with their correspondents on the maternal side. The position of the thus-formed bivalents "in the equatorial plate of the reducing division is purely a matter of chance, that is, that any chromosome pair may lie with maternal or paternal chromatid indifferently toward either pole irrespective of the position of other pairs and hence, that a large number of combinations of paternal and maternal chromosomes are possible in the matured germ products of an individual" (W. S. Sutton, "The Chromosomes in Heredity," Biol. Bull., IV., 5).

II.

The nuclei of the several generations of oögonia have already been described (Fig. 3) and need not again be considered except to note that the two nucleoli or the one after the fusion are always homogenous in structure and stain exactly like plastin bodies. These invariably disappear in the ensuing mitosis. In the youngest oöcytes, a new nucleolus, more rarely two, makes its appearance (Fig. 12, *a*). This body is then quite small and may be situated anywhere within the nucleus; sometimes near the periphery, sometimes on an arm of a chromosomal V or even in the midst of a number of such intersecting arms. It stains intensely at this stage, and shows as yet no trace of vacuolization. In iron-hæmatoxylin, carm-alum, Borel's fluid, etc., it stains more deeply than the chromatin itself and might, if superficially considered, be looked upon as a chromatic nucleolus, as several workers have, on such evidence as this, actually considered it. In Auerbach's fluid, however, the true nature of the body is very clearly brought out. It stains invariably a deep red with the acid fuchsin and shows no trace of the methyl green with which the chromosomes are colored.

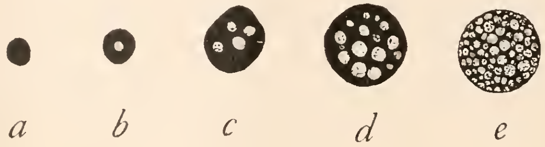


Fig. 12.

As the oöcyte grows, the nucleolus increases in size, and at the same time usually becomes associated with one or more of the chromosomes, and about this period a small vacuole makes its appearance in the center of the body. It stains very slightly and stands out in marked contrast to the darker outer portion (Fig. 12, *b*). From this point onward the course in nucleolar changes is fairly direct and consists in the constant increase of the area of vacuolization until at the end of the period of growth only a minimal portion of the now very large nucleolus shows any stain.

This process of vacuolization may, however, take one of two courses. In the first case, there arise one or more small vacuoles in the immediate vicinity of the first (Fig. 12, *c*), and, with the growth of the nucleolus, the number of these also increases (Fig. 12, *d*), until finally a structure of honey-comb appearance is produced (Fig. 12, *e*). The vacuoles, which fill up the entire space of the nucleolus, are now fairly uniform in size. In the second case the additional vacuoles fuse with the primary one, or the latter may increase in size directly. In either instance, the end result is the same, viz., the central area is much increased in diameter, and by the time the yolk makes its appearance in the egg-cytoplasm only a thin outer rim remains of the deeply-staining portion (Fig. 13, *a*, *b*). Vacuoles may, however, later make their appearance in this outer rim (Fig. 13, *c*), showing very clearly that there is no fundamental distinction between the two types above described.

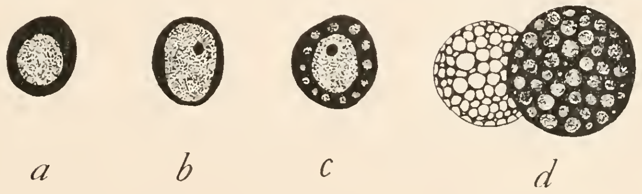


FIG. 13.

In both these groups, moreover, additional nucleoli often arise secondarily, and these pass through the same processes described for the primary body. In some instances these may fuse, giving rise to a compound nucleolus, one part of which is generally, as is shown by the differences in staining reaction, a little further advanced than the other (Fig. 13, *d*). In these types also there are often observed certain individual variations which are of interest in their bearing on the relations between the nucleolus and the chromosomes. A cap of deeply-staining material, showing no sign of vacuoles within it, often covers one part of the structure. This varies in size, from the merest rim to a mass half as large as the fully grown nucleolus itself. In some cases there are two such caps to the same nucleolus (Fig. 14, *a*). The other variation consists in the frequent indentation of the outer rim, which gives the appearance of disintegration on the part of the nucleolus (Fig. 14, *b*). This view is made probable by the presence in such cases of large granules, which lie within the indentation and seem directly to fit into it. These conditions, moreover, are found only in advanced stages of egg-development. Altogether, the appearance very strikingly resembles Fig. 13 of Guenther's work, '03, on the nucleolus in the maturing eggs of the Echinodermata (*Holothuria tubulosa*).

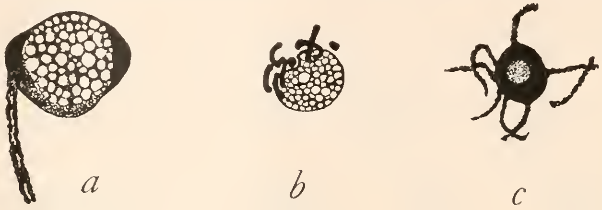


FIG. 14.

This author considers this one of the strongest elements in the evidence, for the chromatic nature of the nucleolus, since the indentation is taken to signify that the nucleolar substance has broken off to become a chromosome. Such a picture as that shown in Fig. 14, *b*, where, in an egg nearly ready for the first maturation, some of the much concentrated chromosomes actually lie within a nucleolar indentation, would most probably be interpreted by Gunther, '03, and some other authors, as very strong evidence in favor of their hypothesis. Indeed, when stained with hæmatoxylin, some of the chromosomes are in such intimate connection with the nucleolus, that all the necessary conditions which their hypothesis demands seem to be realized. Yet nothing can be further from the truth. I have fortunately found such a stage among my Auerbach preparations, and the results dispel any possibility of confusion. The nucleolus, at this point completely vacuolated, stains intensely red and, in most striking contrast, all the chromosomes lying on its side are as intensely green. It is hardly possible that this striking distinction in staining capacity would exist if there were any more intimate connection between the chromatin and the nucleous, than mere apposition. But more conclusive evidence is given by the fact that the characteristic concentration and localization of the chromosomes, immediately before the first maturation division, is, in most instances, passed through without any relation to the nucleolus. In addition, the history of the chromatin in the growing oöcyte is of a nature entirely incompatable with the conception of the authors cited above. As is pointed out in the section on oögenesis (Dublin, '05), the chromosomes persist, in the reduced number, from the period of synapsis up to the time when they enter into the first maturation spindle. At no stage is there a break in this continuous history. At no stage is a chromatin reticulum formed which might, in some way, make the continued observations of the chromosomes an uncertain task. On this point, the evidence appears to be decisive. These indented nucleoli often occur, when the chromosomes are at the highest point of their extension, as threads, and when even the smallest of them would be many times as large as the space out of which they are supposed to arise. We can now understand the true significance of a condition such as is shown in Fig. 14, *c*, where the chromosomes radiate from the nucleolus and seem to originate from it. This is simply a case, rare enough, where the chromosomes in their extension through the nucleus have crossed such a body. It is probable, further, that they have been intimately associated with the nucleolus by the coagulation incident to the fixing process.

In connection with the deep staining cap-formation on the much vacuolated nucleolus, it is very probable that we are concerned with a phenomena similar in all respects to that observed by Obst, '99, in *Limax*, and in other Molluscs, where a nucleolus was found to arise from the chromatin in the process of concentration. These nucleoli arise late in the oöcytic growth and, in contrast with the cyanophilous nucleoli of the early oöcytes, they are erythrophilous. In *Pedicellina*, at the stage of the formation of these caps, the chromosomes are in the process of shortening from long ragged threads to small thick rings and bars of the first maturation mitosis, and it is evident that a considerable part of their substance is lost during the change. Fig. 14, *a*, shows very clearly the apposition of one of these long chromosomes to the cap with the strong probability that the substance of the latter is being increased at the expense of that of the former.

As in *Limax* also, the nucleolus of the *Pedicellina* ovum plays no observable part in the formation of the first polar figure. Its structure, now much reduced, disintegrates and its remains are cast out into the light area with which the spindle is surrounded.

The facts observed appear to harmonize, in the main, with the general conclusions which Häcker, '95, has reached. At no period of egg-development is there any possibility for the origin of chromatin from the nucleoli. Indeed, the only perceptible relation which it may have with the chromosomes, excepting those obviously ascribable to accidental apposition, are those where the former adds to its own substance from the cleavage products of the latter. When the uncertain evidence of such works as those of Guenther and Hartman, and the absolute denial given by Häcker, '02, Miss King, '01, and Janssen, '04, to the results of Carnoy and LeBrun, '97–'99, is considered, then it may be concluded that the true nucleoli may, after all, be of one type, and are to be distinguished from the undoubted chromatin bodies such as those described by Blackman, '03, Wilson, '01, and others which are but temporary aggregations of chromatin unwinding the materials from which they are formed.

It gives me great pleasure to acknowledge my indebtedness to Prof. E. B. Wilson under whose observation this study was made.

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January 6, 1905.

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FOSSIL CEPHALOPODA, DESCRIBED BY HYATT AND CRAGIN, IN THE MUSEUM OF THE UNIVERSITY OF TEXAS.[1]

ANNIE H. PRITCHETT.

In the Second and Third Reports of the Geological Survey of Texas, published in 1890 and 1892 respectively, Professor Alpheus Hyatt described a number of Nautiloids from material collected in Texas under the direction of the then State Geologist, Mr. E. T. Dumble; and in the Fourth Report, Professor F. W. Cragin describes a number of forms, mainly Ammonites. The material described in these three papers represents only a portion, about an eighth, of a rather extensive collection of specimens of these groups, but the portion that is most interesting because of the number of type specimens. Only recently has this material been unpacked and arranged, and because it is the largest collection of the kind in Texas, and because for so many years it has been inaccessible to students, it seemed desirable to publish a short list of the more important specimens. The task has been relatively simple, since in only a few cases had the original labels been lost or misplaced; and in most cases the describers had marked new species with the word "type."

In the following list the name of each species is followed in parentheses by the name of the particular describer, and the volume and page of the published Report.

Nautiloidea.

Temnocheilus conchiferous, type (Hyatt, Second Rep., p. 329).
T. forbesianus McChesney (Hyatt, Second Rep., p. 330).
Tainoceras cavatum, type (Hyatt, Second Rep., p. 341).
T. quadrangulum McChesney (Hyatt, Fourth Rep., p. 402).
Stearoceras gibbosum, type (Hyatt, Fourth Rep., p. 424).
Coloceras globulare, type (Hyatt, Fourth Rep., p. 452).
Glyphioceras cumminsi, type (Hyatt, Fourth Rep., p. 467).
G. incisum, type (Hyatt, Fourth Rep., p. 471).

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[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 66.

Phacoceras dumbli, type (Hyatt, Second Rep., p. 347).

Gastrioceras compressum, type (Hyatt, Second Rep., p. 355).

G. entogonum Gabb (Hyatt, Fourth Rep., p. 472).

Metacoceras walcotti, type (Hyatt, Second Rep., p. 337).

M. inconspicuum, type (Hyatt, Second Rep., p. 340).

Domatoceras militarium, type (Hyatt, Fourth Rep., p. 445).

(The type specimen of *Domatoceras simplex* Hyatt, Fourth Rep., p. 441, appears to have been lost.)

Ephippioceras divisum White and St. John (Hyatt, Second Rep., p. 350).

Solenocheilus collectus Meek and Worthen (Hyatt, Fourth Rep., p. 463).

Paralegoceras iowense Meek and Worthen (Hyatt, Fourth Rep., p. 474).

Nautilus texanus Shumard (Cragin, Fourth Rep., p. 236).

Ammonitoidea.

Placenticeras syrtalis cumminsi, type (Cragin, Fourth Rep., p. 237).

Pulchella bentonianum, type (Cragin, Fourth Rep., p. 239).

Scaphites septum-seriatus, type (Cragin, Fourth Rep., p. 240).

Sphenodiscus dumbli, type (Cragin, Fourth Rep., p. 243).

S. rœmeri, type (Cragin, Fourth Rep., p. 245).

(The type specimen of *Sphenodiscus emarginatus*, Cragin, Fourth Rep., p. 245, appears to have been lost.)

S. lenticularis Owen (Cragin, Fourth Rep., p. 245).

Hoplites rœmeri, type (Cragin, Fourth Rep., p. 234).

H. texanus, type (Cragin, Fourth Rep., p. 235).

Pachydiscus brazoensis Shumard (Cragin, Fourth Rep. p. 236).

P. complexus Hall and Meek (Cragin, Fourth Rep., p. 237).

Crioceras annulatus Shumard (Cragin, Fourth Rep., p. 234).

Schlœnbachia peruviana Von Buch (Cragin, Fourth Rep., p. 242).

S. woolgari Mantell (Cragin, Fourth Rep., p. 243).

S. leonensis anatina Conrad (Cragin, Fourth Rep., p. 241).

Buchiceras inæquiplicatus Shumard (Cragin, Fourth Rep., p. 233).

B swallowi Shumard (Cragin, Fourth Rep., p. 234).

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January 15, 1905.

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